SELECTED CONTRIBUTIONS TO THE LITERATURE OF BLOOD GROUPS AND IMMUNOLOGY

Dunsford Memorial

VOLUME 1. THE ABO SYSTEM

Blood Transfusion Division
US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121

1966

PURPOSE

This series of fundamental research reports from the field of blood group immunology has been prepared for the Fellowship in Blood Banking and Immunohematology for career military personnel. The camera-ready copy and translation of these works were prepared by the Frank C. Farnham Company, Philadelphia, Pa. Certain prime English reports have also been republished because of their extremely limited availability. One very recent report has been included because it so perfectly supplements an older paper on the same topic. It is upon such fundamentals as those set forth in these works that the specialty of blood transfusion therapy has reached its present level of preeminence.

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Brigadier General Colin F. Vorder Bruegge, MC

Commanding

U. S. ARMY MEDICAL RESEARCH LABORATORY

Colonel Robert J. Hoagland, MC

Commanding Officer and Director

INTRODUCTION TO THE SERIES

The translation in 1964 of Arne Gammelgaard's thesis on weak A antigens resulted from our belief that this was of fundamental importance to the study of group A bloods weaker than A_2. Despite its inaccessibility for more than 20 years, this work has been cited in the references of the majority of papers dealing with this subject which have appeared in English during the past decade and more. In retrospect, much of the confusion in classifying the weak subgroups of A might have been avoided if Gammelgaard's data and conclusions had been more widely appreciated. Impressed by the impact of the Gammelgaard experience, and desiring to encourage scholarly pursuit in the Fellowship in Blood Banking and Immunohematology, we have assembled 34 papers and 2 monographs mainly from the older scientific literature. Having thus been faced with the substantial effort and expense of obtaining these translations for our own use, we have been encouraged to make these papers available to workers the world over.

The papers will appear in five volumes. The initial collection, Volume I, dedicated as a memorial to Ivor Dunsford, contains eight titles covering the ABO system. Volume II, containing nine papers, covers the secretion of blood group specific substances and the Lewis system. Volume III contains four papers dealing with the MN and P systems and a monograph by Ludwig Hirszfeld. Volume IV contains six papers and a monograph by Fritz Schiff covering anthropologic and other applications of blood grouping data. Volume V, scheduled for release late in 1967, will commemorate the centennial of the birth of Karl Landsteiner. Six of the seven papers in this volume are by Landsteiner and deal with basic concepts in immunology.

It is hoped that the series of papers will stimulate established workers in the field of immunohematology and provide newcomers to this area a sound indoctrination in this exciting discipline.

FRANK R. CAMP, JR.
Lieutenant Colonel, MSC, USA

FRANK R. ELLIS
Colonel, MC, USAR

CHARLES E. SHIELDS
Lieutenant Colonel, MC, USA

Fort Knox, Kentucky
December, 1966

This Volume of Translations
is
IN MEMORY OF
Doctor Frank Ivor Noel Dunsford
November 30, 1915 - April 23, 1965.

Teacher Scientist Friend

DEDICATION

Ivor Dunsford was born in Somercotes, Derbyshire. At age 16 he entered an apprenticeship in pharmacy, ultimately working as First Assistant with a prominent firm in Southwell, Nottingham. In 1939 he entered Nottingham University (then University College) as a candidate for the Diploma of the Pharmaceutical Society of Great Britain and the London University degree Bachelor of Pharmacy. In 1942, during the World War II interruption of this course for male students, Dunsford joined the National Blood Transfusion Service as a technical bench worker. Rising through the ranks -- from Chief Technician to Senior Scientific Officer -- he served with the Regional Transfusion Center, Sheffield, the remaining 23 years of his life. At the time of his death he had published over 60 papers in addition to his books, thesis, and other articles. Among his many achievements were the following memberships and honors:

Doctorate in Philosophy -- 1957,
Sheffield University Faculty of Medicine

Fellow, Institute of Biology
Member, College of Pathologists
Fellow, Royal Society of Medicine
Founder Member, British Academy of Forensic Science

Member:
British Society for Haematology
Genetical Society
Haematological Society of Great Britain
American Association of Blood Banks

Honorary Member:
Argentine Society of Hematology
Argentine Society of Blood Transfusion

Oliver Memorial Award in 1961

He married the former Margaret Leek in Southwell Cathedral on September 7, 1943. He is survived by his widow and three children, Peter, Ann-Marie, and Roger.

From our initial meeting during the VIth Congress of the International Society of Blood Transfusion in Boston, 1956, there grew a deep and warm friendship. Common interests in the study of group A bloods weaker than A_2 led to frequent correspondence and exchange of specimens. Inevitably his trips to the United States resulted in renewed pleasure and scientific stimulation. One such visit, coupled with his plea for the translation into English of early prime references in the field of blood groups, resulted in the appearance in 1964 of the Doctoral Thesis entitled "On Rare Weak A Antigens (A_3, A_4, A_5, and

A$_1$) in Man" by Arne Gamelgaard. Translation of the Gamelgaard thesis was supported by funds provided by the Walter Reed Army Institute of Research. Copies are still available at modest cost from the Government Printing Office.

The present collection of eight papers covering the ABO system has been supported by funds provided by the United States Army Medical Research and Development Command. This volume may serve appropriately to honor the memory of a contemporary who himself made significant scientific contributions in this area.

FRANK R. CAMP, JR.
Lieutenant Colonel, MSC, USA

Fort Knox, Kentucky
December, 1966

FRANK R. ELLIS
Colonel, MC, USAR

TABLE OF CONTENTS

CONTRIBUTION TO THE KNOWLEDGE OF THE ANTIENZYMATIC, LYTIC AND AGGLUTINATING EFFECTS OF BLOOD SERUM AND LYMPH

Karl Landsteiner

Translation of "Zur Kenntnis der antifermentativen, lytischen und agglutinierenden Wirkungen des Blutserums und der Lymphe". Zentralblatt für Bakteriologie 27: 357-366, 1900.

U. S. ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX KENTUCKY 40121

BLANK PAGE

CONTRIBUTION TO THE KNOWLEDGE OF THE ANTIENZYMATIC, LYTIC AND AGGLUTINATING EFFECTS OF BLOOD SERUM AND LYMPH

Karl Landsteiner

I. Serum Diagnosis of Enzymes

*/357

The studies made by Fermi [1], Pernossi [1], Hammarsten, Hahn [2], Röden [3], Hildebrant [4] and Morgenroth [5] reveal that blood serum has the ability to neutralize the effect of some enzymes. Fermi and Hahn conducted their experiments with digestive enzymes, while Röden and Morgenroth used rennin.

It can easily be understood that it was expected to use this peculiar behavior of serum for investigation of enzymes. Morgenroth [6] hopes to conduct experiments using the effects of serum to show that several active groups can be found in rennet. In a different manner, v. Dungern [7] attempted to utilize the antienzymatic effects of serum by immunizing animals with various microbes and showing that the resulting serum has a specific effect against the bacterial enzymes which he introduced. Therefore, in these experiments we are dealing with a kind of "serodiagnosis" of bacteria, using a roundabout route via their enzymes. It appears that a practical use of this behavior has not yet been found.

The experiments I conducted used serum as an ancillary means for distinguishing those animal enzymes which could not be differentiated in any other way. For the object of my investigation I chose tryptic enzyme (trypsin). I proceeded from the supposition that enzymes of the same name but from different species of animals could be characteristic for each species, as is the case for

---

*/Numbers in the margin indicate pagination of the original foreign text.

the active agents in their serum, the hemoglobins and certain other constituents of red blood cells, as well as for the cells of animals in general.

The perception of regularly occurring characteristics of very similar, initially indistinguishable substances in the different species was aided in part by chemical investigations, however, physiological and morphological deliberations led to the same conclusions. On the basis of experience gained with transplantation experiments and his investigations on the lens structure of the eye, Rabl [8] has recently pointed out the constancy of such differences found in homologous structures of different animal species.

In the case of enzymes, the most apparent method would have been to use /358 the sera of animals previously injected with enzymes. This sera would be similar to that which Morgenroth [9] produced by administering rennin to goats; a serum which was very active toward rennin.

Morgenroth himself has pointed out the difficulties which may be encountered in these experiments. In the case of rabbits, e.g., he did not succeed in rendering the serum active toward rennin. I experienced similar failures with the injection of large quantities of trypsin into rabbits; even after several injections there was no increase of the antitryptic effect.

I then used normal serum from different species of animals and let it act on pancreatin obtained from different species. With the difference of the sera and the similarity of the enzymes, we can, under these circumstances, expect a proportionality of the effect of sera A, B, C... on trypsins a, b, c...; with different sera and different enzymes, we may expect disproportionality. The experiment seemed to indicate differences in the enzymes, since combinations of sera and enzymes with non-proportional effects could be found.

Rabbit serum inhibited the digestion of gelatine by rat trypsin more than guinea pig serum, while, inversely, the same guinea pig serum had a stronger effect on canine trypsin. Experiments with rabbit serum and bovine serum and with rat tryrsin and bovine trypsin yielded analogous results. The fact that the strength of the antienzymatic effect varies in various individuals of one animal species, makes evaluation of these experiments difficult. However, in spite of this variance, there are certain constant differences in the sera of different species of animals.

Because of the complicated relationship of the interacting agents, it is desirable to examine the production of specific sera.

II. Occurrence of Antienzymatic Substances in the Body

In order to determine the origin of bactericidal substances in the blood serum, investigations were conducted with the purpose of detecting these substances in the tissues. Only in the case of polynuclear leukocytes were these investigations successful. This is also evident from the most recent studies [Moxter]. It seemed desirable to extend the investigations to those antienzymatic substances, whose behavior offers certain analogies to bactericidal substances, as e.g., by artificial intensification of their effect [v. Dungern: 10; and Morgenroth: 10].

Indications as to the reaction of trypsin with fresh organ pulp were made by Fermi and Pernossi [11]. They determined that freshly triturated organs (liver, spleer and muscle from guinea pigs) during a 24-hour digestion period, destroy, just as fresh blood, the effectiveness of a trypsin solution. This effect could be eliminated by heating the organs.

I proceeded in the following manner: before letting the pulverized /359

organs (liver, kidney. spleen) of guinea pigs react with trypsin, I washed them of the strongly antitryptic serum by repeated washing with NaCl solution (0.6%) and centrifugation. Under these circumstances, the antitryptic effect of the organs or of their extracts could not be clearly established. Extracts from polynuclear leukocytes were as ineffective as the organ extracts. However, I found an antitryptic effect in muscle serum, which was prepared in the following manner: A rabbit was bled to death and the blood vessels were then washed of blood by injecting large quantities of NaCl solution. A part of the muscle tissue was very finely diced and pressed in a handpress. The muscle serum that was thus obtained only impeded liquidation of the gelatine if an ample amount of a mixture of gelatine and trypsin was added*. Albumin from a chicken behaved in the same manner.

The question discussed is connected to the problem of the manner by which animal tissues are protected from the effect of the enzymes they contain.

Fermi [12] and Matthes [13] have recently formulated the thesis that living cells are completely resistant to enzymes, while Hahn [14] has pointed out the possible connection of this resistance to the antienzymatic characteristics of the serum. Thus, we are either dealing with humoral or with cellular immunity of the tissues to enzymes. Determination as to which of the two possibilities is applicable or whether both factors act together is not possible at this time since in the digestion experiments on living tissue of higher animals, the blood could not be excluded, and because, on the other hand, experiments on isolated cells (amoeba, mushroom, plant seeds, insect larvae) do not permit any con-

---

*The resistance of the muscles to digestion may be related to this; also Matthes [loc. cit., p. 355] and the repetition of his experiment according to Claude Bernard.

clusion as to the behavior of the tissue of higher animals. I could not convince myself of the resistance of red blood cells, at least not if artificial tryptic digestion fluid was used. The presence of antienzymatic substances in the cells of liver, kidney or spleen cannot be proven with simple extraction.

III. Distribution of Bactericidal Substances in the Body Fluids

Information regarding the bactericidal effect of the lymph fluid does not always agree. I found, as did most other investigators, that lymph from the thoracic duct of dogs is able to kill bacteria. To attest such behavior, I used Vibrio cholerae. Similar to earlier experiments, the effects of the lymph were less strong than those of blood serum.

For comparison, I tested the effect of the duct lymph and lymph from the extremities of a dog. In order to collect this lymph in as pure a state as /360 possible, I used a method similar to the one described by Paschutin [16] and Emminghaus [17], i.e., I inserted cannulas into the lymph ducts of the dog's extremities. Concerning the bactericidal effect of the lymph in the peripheral areas, Hamburger [18] has obtained positive results. However, in a number of other studies we find indications of ineffectiveness of the lymph which collects upon the occurrence of edema in the peripheral parts of the body. Such experiments dealing with the ineffectiveness of cell-free edematous lymph have often been used to strengthen the relationship between the antibacterial effect of blood and its leukocyte content.

In my experiments, the lymph from dog extremities had a bactericidal effect on Vibrio cholerae. This characteristic could be tested by the inoculation of gelatine plates when compared with fresh serum heated to 60°C, and it could also be shown if the lymph was used to activate heated cholera serum, in

which I observed the alteration of the vibrions into small globules. Of course, the lymph contained a few polynuclear leukocytes.

The effectiveness of this lymph was considerably less pronounced than that of the duct lymph, and it could only be shown if small amounts of bacteria were used. This behavior could be attributed to the fact that either the lymph of different organs contains different quantities of bactericidal agents, or that the quantity of these agents increases during passage through the lymphatic glands. The second supposition, which would correspond to the relationship between lymphatic glands and leukocyte production, was tested by comparing the lymph before and after passage through the glands.

For this purpose, I inserted a cannula into one of the two large lymph vessels on the hind leg of a dog. The lymph of these vessels had not yet passed through a gland [Emminghaus]. A second cannula was inserted into the large lymph vessel of the thigh of the other hind leg. This lymph vessel can be found in the sheath of the great femoral vessels; it receives lymph from the lymph nodules of the popliteal fossa. In other experimental animals, I used only one hind leg by first collecting lymph from a central and then later from a peripheral area.

The differences found while testing the bactericidal effects of the samples were too negligible to warrant the assumption that the lymph glands do exert an influence.

In order to explain the pronounced difference which I observed between the lymph of the thoracic duct and that from peripheral lymph vessels, it is important to investigate the diffusion of substances from the inner organs into the thoracic duct.

IV. The Chemical Behavior of Lysins, Agglutinins* and Antienzymes /361

Bovine serum was diluted six-fold by distilled water containing an ample amount of carbonic acid; the globulin precipitate was separated, and the liquid was divided into a small portion (A) which contained the globulin, and a greater portion (B) which was free of the precipitate (ratio of the liquid volumes = 1:4). NaCl solution (6%) was added to both portions until the salt content was about 0.6%. During this procedure the globulin precipitate dissolves, with only a faint turbidity remaining. Now (A) had a much stronger agglutinating effect on the red blood corpuscles of guinea pigs than the globulin-poor solution (B). The agglutinating effect of globulin can still be shown if the liquid volumes of the two solutions are kept the same. The effect can still be found in globulin solutions which have been rinsed with water several times, in the cold. A large part of the agglutinating substance passed into these globulin precipitates, which were produced either by dialyzation of bovine serum or by precipitating with ammonium sulfate. This result is related to the observations of Winterberg [19], who, like Widal and Sicard [20], found that most substances which precipitate globulin also precipitate the specific typhus agglutinins. Winterberg, who used different precipitating agents, found a difference in their ability to precipitate globulins and agglutinins, a relationship which must still be tested for normal agglutinins, probably the mother material for these

---

*The serum of healthy humans not only has an agglutinating effect on animal blood corpuscles, but also on human blood corpuscles from different individuals. It remains to be decided whether this phenomenon is due to original individual differences or to the influence of injuries and possible bacterial infection. I observed this behavior as especially pronounced in the case of blood from severely ill patients. This phenomenon could be related to the dissolving capacity of serum for blood corpuscles in the case of various diseases, as it was described by Maragliano (10th Congress of Internal Medicine, 1892).

specific substances.

When both solutions (A) and (B*) were tested as to their reaction with trypsin, it was found that (A) did not have a stronger antitryptic effect than (B), from which it is evident that the antitryptic effect is for all practical purposes independent of the globulins. The globulins which were obtained by means of dialysis yielded similar results. If bovine serum is precipitated with an equal volume of ammonium sulfate solution, and after filtering, with crystallized ammonium sulfate, the solution of the globulin precipitate which was produced first, is ineffective toward trypsin, whereas the solution of the albumin precipitate has an antitryptic effect, before as well as after removal of the ammonium sulfate by means of dialysis.

If solutions (A) and (B) are tested as to their content of blood cell dissolving substances (red blood cells from guinea pigs), it is found that both solutions are rather ineffective. Buchner [21] proceeded in a similar man- /362
ner during his investigation of bactericidal substances and found that those solutions which contain primarily albumin have a stronger effect than those which contain globulin. As far as the globulicidal substances are concerned, he found that the primarily globulin-containing solution from a sodium sulfate precipitate of canine serum is able to dissolve blood cells. I, too, found this characteristic in the case of precipitates produced by carbonic acid using bovine serum, however, the effect of the concentrated solution was very small when compared to the original serum, therefore, the effect could merely be due to the substances which clung to the precipitate. With this experimental

---

*Both solutions inhibit the effect of abrin on red blood corpuscles. When compared to the original serum, both solutions have a rather weak effect on the cleavage of hydrogen peroxide.

arrangement, most of the globulicidal effect is lost, regardless of whether the precipitation is caused by dilution and carbonic acid or by dialysis. The probable reason for this behavior is the destructive effect of water, which was pointed out by Buchner. Therefore, I conducted new experiments, where I again precipitated the globulins with carbonic acid, but where I did not dilute with water, using instead a solution of grape sugar (glucose, 5%). Using this method, it is possible to maintain the globulicidal effect, and it could now be shown that the globulin-rich liquid has no greater effect than the globulin-poor solution, in fact, in several cases the latter seemed to have a more pronounced effect. Accordingly the globulins would be rather unimportant for the dissolution of foreign blood corpuscles in the serum. The sensitizing substances of Ehrlich and Morgenroth [22] were found, in the case of the dialyzed bovine serum, in the still-dissolved portion but not in the precipitated globulin. If this solution was mixed with NaCl, it was not until it contained about 0.6% NaCl that it rendered the blood corpuscles of guinea pigs sensitive to the alexins of guinea pig serum, just as heated serum did in the experiments cited above.

Vienna, February 10, 1900.

REFERENCES

1. Fermi and Pernossi: Zeitschr. f. Hyg., Vol.XVIII and in other places.
2. Hahn: Berl. klin. Wochenschr., p. 499, 1897.
3. Röden: cited by Hahn.
4. Hildebrandt: Virch. Arch., Vol. CXXXI.
5. Morgenroth: Centralbl. f. Bakt., Vol. XXVI, Nos. 11 and 12.
6. Morgenroth: loc. cit.
7. v. Dungern: Münch. med. Wochenschr., p. 1040, 1898.
8. Rabl: 71st Congress of German Natural Scientists.
9. Morgenroth: loc. cit.
10. v. Dungern: loc. cit.; Morgenroth: loc. cit.
11. Fermi and Pernossi: loc. cit.
12. Fermi: Centralbl. f. Phys., p. 657, 1895; and p. 57, 1896.
13. Matthes (bibliography): Ziegler's Beitr., Vol. XIII; see also E. Freund: Wien. klin. Wochenschr., p. 637, 1897.
14. Hahn: loc. cit.
15. Löwit: Ziegler's Beitr., Vol. XXII, p. 172; Neisser: Zeitschr. f. Hyg., Vol. XXII, p. 20; and Meltzer and Norris: J. of Exp. Med., Vol. II, p. 701.
16. Paschutin: Arb. a. d. phys. Anst. of Leipzig, p. 197, 1872.
17. Emminghaus: ibid., p. 51, 1873.
18. Hamburger: Virch. Arch., Vol. CLVI, p. 329.
19. Winterberg: Zeitschr. f. Hyg., Vol. XXXII, p. 374.
20. Widal and Sicard: Ann. de l'Inst. Pasteur, p. 353, 1897.
21. Buchner: Arch. f. Hyg., Vol. XVII, pp. 112 and 138.
22. Ehrlich and Morgenroth: Berl. klin. Wochenschr., p. 481, 1899; see also Moxter: Centralbl. f. Bakt., Vol. 26.

AGGLUTINATION PHENOMENA IN NORMAL HUMAN BLOOD

Karl Landsteiner

Translation of "Über Agglutinationserscheinungen normalen menschlichen Blutes". Wiener Klinische Wochenschrift 14: 1132-1134, 1901.

U. S. ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX KENTUCKY 40121

BLANK PAGE

AGGLUTINATION PHENOMENA IN NORMAL HUMAN BLOOD

Karl Landsteiner

Some time ago, I observed and reported [1] that the blood serum of */1132 normal humans can sometimes clump the red blood cells of other healthy individuals. At that time, I was under the impression that the ability of the blood serum to clump foreign red cells was especially pronounced in the case of certain diseases, and thought that it could be connected with the strong lytic ability of pathological sera, as was observed earlier by Maragliano [2], since agglutination and lytic abilities often, if not always, undergo parallel changes. The reaction studies conducted by Maragliano would not be equated with the hemolytic reaction studies which are now so frequently conducted, because the addition of NaCl (and not heating) until a normal NaCl content is reached, eliminates the lytic ability of the sera. Maragliano himself distinguishes his observations from the phenomenon of Landois (hemolysis by serum foreign to the species), since in Maragliano's case the hemoglobin is not only dissolved but also destroyed. One significant difference between my observations and those of Maragliano is that in his case the serum only affects the blood cells taken from the same individual and that the reaction is only successful if conducted with pathologic blood. However, my observation pointed up clear differences between the blood serum and the blood cells of apparently completely healthy persons.

According to his descriptions and to his pictures, the observation of Shattock [3] belongs in this context, even though he only found the reaction in

---

*/Numbers in the margin indicate pagination of the original foreign text.

cases with febrile diseases, not in normal blood. Shattock relates this reaction to increased coagulability and rouleau formation in the febrile blood.

According to Ehrlich and Morgenroth [4], the clumping of human blood /1133 by human serum, which will be discussed further, should be designated as isoagglutination. These two researchers described their experiments shortly after my publication appeared. They described experiments in which they succeeded, by means of homogeneous blood injection, in producing isolysins and isoagglutinins, i.e., sera which would act on cells of the same species. These very detailed experiments confirm the unexpected occurrence of clearly distinguishable differences in the bloods of one species of animal, partly because of the different circumstances in the testing of the individual experimental animals.

In the study by Ehrlich and Morgenroth, the phenomenon of isolysis (isohemolysis) is subjected to a more detailed discussion from the viewpoint of Ehrlich's theory.

Since the publication of the reports made by Shattock and myself, a number of investigators have studied the behavior of isoagglutination in humans. The evaluation of those studies* which consider this reaction to be specific for a certain disease, is valueless as is apparent by the fact that this reaction occurs among healthy persons. Other studies even record observations on intensity and frequency of the reaction in cases of disease.

In various forms of anemia, Donath [5] found the phenomenon more frequently than in healthy persons, but not every time. Ascoli [6] observed the phenomenon in healthy persons, but noted more intensity in diseased ones. Eisenberg conducted observations on both healthy and diseased persons. As other authors,

---

*For other references, see article by Eisenberg: Wiener klinische Wochenschrift No. 42, 1901.

he found that the reaction is frequent in cases of disease, and constitutes rather an exception in healthy individuals. This result is not in agreement with my findings*.

Since, in the above-mentioned publications I gave only brief descriptions, I will indicate below the results of some recently conducted experiments. The tables are quite simple. Approximately equal quantities of serum and red blood cell suspension (about 5%) were mixed in NaCl solution (0.6%) and observed in a suspended drop preparation or in test tubes (the plus sign designates agglutination).

TABLE I. CONCERNING THE BLOOD OF SIX APPARENTLY HEALTHY MALES.

Serum from:	Blood cells from:					
	Dr. St.	Dr. Plecn.	Dr. Sturl.	Dr. Erdh.	Zar.	Landst.
Dr. St.	-	+	+	+	+	-
Dr. Plecn.	-	-	+	+	-	-
Dr. Sturl.	-	+	-	-	+	-
Dr. Erdh.	-	+	-	-	+	-
Zar.	-	-	+	+	-	-
Landst.	-	+	+	+	+	-

A fourth, similar table, concerning the sera of Table II, combined with the blood cells of Table I, and certain other tested sera, e.g., from one case

*Although Eisenberg attacks the results of my work, and simultaneously confirms them as far as the blood of the patients is concerned, he mentions my work only in the bibliography but does not refer to it at all in the text.

TABLE II. CONCERNING THE BLOOD OF SIX APPARENTLY HEALTHY PUERPERAE.

Serum from:	Blood cells from:					
	Seil.	Linsm.	Lust.	Mittelb.	Tomsch.	Graupn.
Seil.	-	-	+	-	-	+
Linsm.	+	-	+	+	+	+
Lust.	+	-	-	+	+	-
Mittelb.	-	-	+	-	-	+
Tomsch.	-	-	+	-	-	+
Graupn.	+	-	-	+	+	-

TABLE III. CONCERNING THE BLOOD OF FIVE PUERPERAE AND SIX PLACENTAE (BLOOD FROM THE UMBILICAL CORD).

Serum from:	Blood cells from:					
	Trautm.	Linsm.	Seil.	Freib.	Graupn.	Mittelb.
Lust.	+	+	-	-	-	+
Tomsch.	-	-	+	-	-	-
Mittelb.	-	-	+	-	-	-
Seil.	-	-	+	-	-	-
Linsm.	+	+	+	-	-	+

of hemophilia and one case of purpura, showed perfectly corresponding regularities and could therefore be omitted. During the investigation of ten other normal persons (using 42 combinations) similar conditions were found.

These experiments show that my data did not have to be corrected. All of the 22 investigated sera of healthy adults yielded this reaction. The result would obviously have been different if I had not used a number of different blood corpuscles for testing purposes.

Halban [7], Ascoli and, most recently, Eisenberg, have already pointed out the variable resistance of the blood cells to this reaction. This difference can also be seen from the above tables. Moreover, the behavior of the 22 investigated blood samples showed a curious regularity. If we do not take into account the few blood serum investigations on fetal placenta blood which did not show agglutination (Halban also found that fetal blood seldom has an agglutinating effect), the sera in most cases could be separated into three groups:

In a number of cases (group A), the serum reacts with the cells of another group (group B), but not with those of group A; these A cells are acted upon in the same manner by serum B. In the third group (C), the serum agglutinates the corpuscles of A and B, while the red cells of group C are not acted upon by sera from A and B.

According to the customary terminology, one can say that in these cases there must be at least two kinds of agglutinins present, the one in A, the other in B, and both together in C. Naturally, the corpuscles must be considered insensitive to the agglutinins which are present in the same serum.

It cannot be denied that a postulate on the occurrence of a few different agglutinins in the investigated cases sounds quite strange, even though the experiments on isolysins made by Ehrlich and Morgenroth yielded similar results. It would be more satisfactory if continued observations would lead to a different interpretation.

However, the investigations indicate that attention should be paid to these

regularities in pathological cases.

Eisenberg attributes the formation of agglutinins to the resorption of constituents of the red blood corpuscles. This idea is not new, since Halban and Ascoli have already presented it as a possible explanation. I did not mention this explanation at an earlier time, as I had not succeeded in inducing autoagglutination in animals by injection of their own dissolved red blood corpuscles.

I do not believe Ehrlich has reported any positive results in this area, however, Ascoli does have positive, but not constant results. Halban points to the difficulties with the mentioned postulate. For, according to this postulate, the formation of naturally occurring hemagglutinins as well as the normal agglutinins which act on bacteria, would require two different explanations.

Furthermore, my experiments show that the different sera do not have /1134 identical effects with regard to agglutination. If it is believed that the sera owe their agglutinating ability to a kind of auto-immunization caused by cell constituent resorption, one must still assume the existence of individual differences, in order to interpret different sera. The blood corpuscles do indeed show a differing behavior, even already in the fetal blood (see Table III). If we assume the differences of the sera and of blood corpuscles, it is just as easy (or difficult) to understand agglutination within one species, as agglutination by a serum foreign to the species. However, the above-mentioned explanation cannot be excluded, moreover, if the still-unrefuted experiments of Ascoli are correct, it would be hard to circumvent. It would have to be assumed that the physiological decomposition of the cells of body tissue was the generative source of active serum components.

In order to eliminate the opinion that past pathological processes are of any significance, I would consider the experiments on the blood of infants and

of animals to be of some importance. Halban's experiments also contradict the existence of such a relationship.

The kind of agglutination described can also be caused by serum which has been dried and then immediately dissolved; I succeeded in producing this kind of agglutination with the solution of a dried drop of blood, which had been kept for 14 days on a piece of linen cloth. Thus the reaction may possibly be used in some cases for the identification, or better, for the recognition of unidentified blood samples, e.g., for forensic purposes, unless rapid variations of the agglutinating ability should be found, which would prevent this application. However, the 6 sera of Table I showed the same behavior when the second sample was taken, as the samples taken nine days earlier*.

Finally, it should be mentioned that the described observations permit the explanation of varying consequences resulting from therapeutical blood transfusions.

---

*Dr. Richter and I plan to investigate the reliability of this method.

REFERENCES

1. Landsteiner: Centralblatt für Bacteriologie 27: 361, February 10, 1900.
2. Maragliano: XIth Congress for Internal Medicine, Leipzig, 1892.
3. Shattock: Journal of Pathology and Bacteriology, February, 1900.
4. Ehrlich and Morgenroth: Berliner klinische Wochenschrift, 1900.
5. Donath: Wiener klinische Wochenschrift, No. 22, 1900.
6. Ascoli: Münchener medizinische Wochenschrift, p. 1229, 1901.
7. Halban: Wiener klinische Wochenschrift, No. 24, 1900.

THE ISOAGGLUTININS IN THE SERUM OF HEALTHY AND SICK HUMANS

Alfred von Decastello and Adriano Stürli

Translation of "Über die Isoagglutinine im
Serum gesunder und kranker Menschen".
Münchener Medizinische Wochenschrift
26: 1090-1095, 1902.

U. S. ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX KENTUCKY 40121

BLANK PAGE

THE ISOAGGLUTININS IN THE SERUM OF HEALTHY AND SICK HUMANS

Alfred v. Decastello and Adriano Sturli

Landsteiner [1] and Shattok [2] have pointed out at the same time, */1090
although independently of each other, that the serum of some humans has the capacity to clump the red blood cells of other persons, i.e., that these sera contain, in the terminology of Ehrlich, isoagglutinins. The attention of the investigators was, of course, immediately directed to the question of whether the occurrence of this characteristic is connected to a certain pathological symptom. Landsteiner himself had indicated that this phenomenon is especially clearly developed in cases of severe illness, whereas Shattok understood it to be a characteristic peculiar to febrile blood and connected it with increased rouleau formation.

In fact, Donath [3], Ascoli [4], Camus and Pagniez [5], and Eisenberg [6] /1091
all gained the impression from their studies that this characteristic is especially clear in cases of anemia, cachexia, and fever (in connection with tuberculosis), although all of the investigators stress the inconstancy of the phenomenon. Only Lo Monaco and Panichi [7] and, confirming their findings, Grixoni [8], consider isoagglutination to be a symptom characteristic for malaria blood. On the other hand, Grünbaum [9] indicates that in the case of various infectious diseases, the blood serum of those patients is able to agglutinate the blood of healthy people, or of those that are suffering from different diseases, but not of those that are suffering from the same disease. However, the relation of the agglutinating capacity of blood to various disease

---

*/Numbers in the margin indicate pagination of the original foreign text.

states was questioned by the work of Halban [10], who observed the same characteristic in a series of neonates (placenta blood). Halban, and before him Ascoli, pointed out the fact that some sera cause agglutination only in certain blood samples, while failing to do so in other cases. These various contradictions were substantially clarified by Landsteiner [11], who, by allowing the same serum to react with a series of blood samples, succeeded in showing that every serum has certain agglutinating capacities. At the same time, his tables showed that there existed a peculiar regularity, permitting him to divide the investigated blood samples into three groups, which Landsteiner characterized with the following words: "In a number of cases (group A), the serum reacted with the blood cells of another group (B), but not with group A, whereas the blood cells of group A are influenced in the same manner by serum B. In the third group (C), the serum agglutinates the blood cells of A and B, whereas the blood cells of C are not influenced by the sera A and B.

According to the usual mode of expression, one can say that in these cases at least two kinds of agglutinins are present, one in A, the other in B, and both together in C. Naturally, the corpuscles must be considered to be insensitive to the agglutinins which are found in the same serum."

The purpose of the present study was, on the one hand, to test more healthy individuals for the purpose of investigating the general validity of this typical behavior, and on the other hand to investigate these conditions in a greater number of disease instances. The tests were conducted in suspended drops, by mixing a drop of the serum with the same quantity of a 5% suspension of the blood cells in an .85% NaCl solution. Sometimes we added a correspondingly small quantity of cleansed, centrifuged blood cell paste to the serum, to avoid dilution of the latter.

In the positive cases, agglutination usually begins with great speed and distinctness: in several seconds, the blood cells are already clumped into some large and some small, dense clumps. Even in the case of weak sera, the phenomenon becomes apparent in the course of a few minutes.

The reaction can be initiated or accelerated by shaking or waving the preparation, since only then do the blood cells establish contact with each other. As with other investigators, in the beginning we continued to observe the samples for several hours. However, we were soon convinced that in the case of undiluted sera, if agglutination did not occur within the first few minutes, we could never observe a positive reaction at a later time. On the other hand, if the serum is diluted, e.g., to evaluate its strength, it may take ¼ to ½ hour for agglutination to commence. If at all, the agglutinating substance is apparently present in sufficient quantities (in adults) to cause a prompt reaction.

We must mention one circumstance which in the beginning, led us and probably other investigators to an error. Namely, the occurrence of more or less apparent rouleau formation, which, at a low level of magnification, can appear as agglutination and which was considered together with agglutination by Shattok, Ascoli and Eisenberg. As Ascoli has already emphasized, two things are prerequisite for rouleau formation: on the one hand, the unchanged biconcave shape of the blood cells and on the other, a suitable medium (in isotonic NaCl solution, even the best preserved blood cells never form rouleaus). Serum is the medium which permits the adherence of the blood cells. Many experiments convinced us that even well preserved erythrocytes have this characteristic, regardless of the serum in which they appear. However, there are gradual differences among the single sera.

The serum of several cases of chlorosis, whose blood preparation showed only insignificant rouleau formation, exhibited this characteristic only to a very slight degree, even with different blood suspensions. The same was true for the serum of a case of hydremic nephritis. However, the serum of a case of pernicious anemia showed lively rouleau formation in a suspension, although nothing could be detected in the native preparation. The latter phenomenon was undoubtedly due to the severe poikilocythemia, whereas the characteristic under consideration remained the same in the serum.

In the case of partial deformation of the erythrocytes in the NaCl solution, one can observe here and there, single small clumps instead of symmetrical rouleaus, so that there is strong similarity to agglutination even under considerable magnification. However, if the same suspension is tested with the same serum about 1 or 2 days later, when the swelling of the "stramonium" shape of the erythrocytes has progressed even further, one will observe no trace of clumping.

In contrast, the genuine agglutination of the blood cells is entirely independent of their shape, and can be observed in older suspensions with the same rapidity as in fresh ones. Further, if rouleau formation was identical to weak agglutination, dilution of a clumped serum would eventually lead to rouleau formation, which, however, is not the case. If the agglutinative capacity is removed from a serum in a way which will be discussed later, it nevertheless maintains the capacity to effect rouleau formation in any fresh blood suspension. Therefore, these two phenomena must be clearly separated.

A second source of error is bacterial infection of the blood suspensions. Through the work of Kraus and Clairmont [12] we know that some bacterial poisons have the ability to agglutinate and to dissolve erythrocytes. Older blood

suspensions should be tested before using them as to whether there is no clumping due to bacterial action.

In our own investigations, we proceeded by allowing the sera to be tested to react with 12 different blood samples (from healthy and sick humans). We thus compiled the following table, in which the "+" designates the positive reaction.

TABLE I.

	Serum from:	Blood Suspensions of:											
		Group C (= A+B)				Group A						Gr. B	
		Dr. Dec.	Dr. J.	Wärterin R.	Wärterin F.	Dr. St.	Dr. Fl.	Dr. Klf.	Typhus 1	Typhus 2	Hemophilic	Dr. M.	Typhus 3
Gr. C (= A+B)	Dr. D.	0	0	0	0	+	+	+	+	+	+	+	+
	Dr. J.	0	0	0	0	+	+	+	+	+	+	+	+
	Wärterin R.	0	0	0	0	+	+	+	+	+	+	+	+
	Wärterin F.	0	0	0	0	+	+	+	+	+	+	+	+
Group A	Dr. St	0	0	0	0	0	0	0	0	0	0	+	+
	Dr. Fl.	0	0	0	0	0	0	0	0	0	0	+	+
	Dr. Klf.	0	0	0	0	0	0	0	0	0	0	+	+
	Typhus No. 1	0	0	0	0	0	0	0	0	0	0	+	+
	Typhus No. 2	0	0	0	0	0	0	0	0	0	0	+	+
	Hemophilic	0	0	0	0	0	0	0	0	0	0	+	+
Gr. B	Dr. M.	0	0	0	0	+	+	+	+	+	+	0	0
	Typhus No. 3	0	0	0	0	+	+	+	+	+	+	0	0

etc.

It is clear that this table agrees completely with Landsteiner,

inasmuch as there is a separation of the sera, as well as the blood corpuscles, into three groups. In this way we initially tested the sera of 50 cases (both healthy and sick) using the same blood suspensions. Since the result was /1092 always the same, later we used only 6 test bloods, two from each group, frequently exchanging the representative types. A total of 174 cases were investigated:

8 neonates (placenta blood);
11 children up to 6 months of age;
21 children of ½ to 14 years; and
134 adults.

If we initially disregard the neonates and the very young children, whose characteristics will later be discussed in detail, the study of a total of 155 persons over 6 months old, with an exception of 4 cases, showed the presence of agglutinating substances in the serum as well as strict division into 3 groups: serum A agglutinated blood B, and, conversely, serum B agglutinated blood A; serum C agglutinated blood A, as well as blood B, yet blood C was neither influenced by serum A, nor by serum B. The four exceptions to this rule behaved in the following manner: the serum reacted with none of the blood samples and thus could not contain any agglutinins, whereas the erythrocytes were agglutinated by all the other sera, thus not resistant to any type of serum, as was the case in the other persons.

Of these 155 cases, 34 (with 1 exception) were healthy at that time and 121 (with 3 exceptions) were ill. Therefore, there is no relation between the agglutination type of blood and any pathological condition.

Even with all the healthy individuals we studied, we checked their personal history as to previous illnesses, especially infectious ones, but could not establish any relation to the blood types.

TABLE II:

CONTAINING 121 DISEASE CASES, OF WHOM 21 WERE CHILDREN AGED 1/2 TO 14 YEARS, AS WELL AS 100 ADULTS

No.	Diagnosis	Type of Serum A	B	C	No.	Diagnosis	Type of Serum A	B	C
8	croupous pneumonia	2	2	4	2	catarrhal icterus	2	-	-
3	fibrinous bronchitis	2	-	1	1	infectious enteritis	-	-	1
5	abdominal typhus	2	1	2	2	perityphlitis	1	1	-
1	acute articular rheumatism	1	-	-	1	Cirrhos. heptat. alcolica c. ictero	-	-	1
20	Tuberculosis:				1	Cirrhosis cholelithias cum ictero	1	-	-
	pulmonary	4	4	3	1	Lues hepat. c. nephrit. chron. interstit.	1	-	-
	peritoneal	-	-	4	1	Ecchinococc. hepat.	1	-	-
	intestinal	-	-	2	1	acute nephritis	-	1	-
	meningitic	-	1	-	8	chronic nephritis	2	3	3
	scrofulous	-	-	2	2	pyelitis	1	-	1
7	chlorosis	1	3	3	1	erythromelalgia	-	-	1
4	pernicious anemia	1	-	3	1	ischias	-	-	1
1	(same)	without type			1	tabes	-	-	1
6	leukemia (myelogenous)	4	1	1	2	chorea	-	1	1
2	pseudoleukemia	1	-	1	1	Basedow's disease	-	1	-
1	hemophilia	1	-	-	1	syringomyelia	1	-	1
2	purpura simplex	2	-	-	1	hemiplegia	without type		
10	Carcinoma:				1	manual contrature	1	-	-
	ventricular	3	1	3	2	hysteria	2	-	-
	intestinal	-	-	2	3	rachitis	1	1	1
	uterine	1	-	-	2	prurigo	2	-	-
1	abdominal lymphosarcoma	-	-	1	1	eczema	1	-	-
5	vitium cordis	4	-	1					
1	myocarditis	-	-	1					
1	bronchial asthma	-	1	-					
1	ventricular ulcer	-	-	1					
1	(same)	without type				Sum	48	23	47
2	ventricular atonia	1	-	1					
2	enteroptosis	1	1	-					

However, it is not impossible that some diseases, especially those which alter the albumin content of the serum, may influence the intensity of the agglutinating capacity. Intensive studies in this direction have not yet been undertaken.

The 3 serum and blood cell types do not occur with the same frequency.

Out of 34 healthy adults, 10 were of type A, 4 of type B and 19 of type C, and one case (a 20-year-old woman, nursing at the time) was found to be atypical, in the manner described above. The portion of pathological cases in these 3 groups is evident from the preceding table.

Thus, out of 121 pathological cases, 48 were of type A, 23 of type B and 47 of type C. In 3 cases no agglutinin was found in the serum and the blood cells were agglutinated by every serum. It can be seen that one of the two simple types (B), is found much less frequently than the two others (A and C), and further that sick people exhibit the same characteristics as healthy ones, and also that in several cases of the same disease we could usually observe all three types. The exceptions consisted merely in the lack of agglutinin.*

From these facts we can conclude that there occur two isoagglutinins in human serum, in some sera only one, in others both together. This interpretation also seemed most probable to Landsteiner, although he says "the assertion that there are only a few different agglutinins sounds rather strange." Indeed, the occurrence of numerous agglutinating substances or just a single one would be much less strange than the occurrence of just two; yet, this assumption cannot be avoided. Aside from the described, regular behavior, the following

---

*During the printing of this study we had the opportunity to examine 2 cases of fresh malaria (tertiana), and contrary to the indications of Lo Monaco and Panichi and Grixoni, found completely typical behavior of the serum (type B and C).

facts confirm this assumption:

If a serum of group A is mixed with a sufficient quantity of group B blood cells and the mixture centrifuged, after a time, serum A not only loses the ability to agglutinate fresh blood from the same person of group B, but also to agglutinate any blood of this group. Conversely, the same is true for a group B serum and an A blood. However, if a C serum is mixed with an A blood, the agglutinating power is lost only for this group, but not for group B (the same is again true for B). If serum A and serum B are mixed, the mixture agglutinates the blood cells of A and B. If serum C is mixed with serum A and serum B, the effect is, as is to be expected, the same as with C alone.

Even if one doubts that he is dealing with two different agglutinins, the two must nevertheless be chemically related, analogously structured bodies, since their effect is like those of pendants. This suggests the possibility that we are dealing with a chemical relationship (in the sense of iso- or polymerism) such as with substances having the same molecular formula, where one can be dextrorotatory and the other levorotatory. With regard to the frequency of the single types, one could assume that the agglutinin of group A represents the basic substance, which often occurs with a chemical modification (double type C), whereas in less frequent cases, the chemical modification is found by itself (type B).

Concerning the intensity of the effect, there are differences among various sera, as well as in the resistance of blood cells to different sera.

For a serum of the double type in physiological NaCl solution, we obtain the following limiting values for different blood cell suspensions.

Dr. Kl, typhus No. 1, Hemophelia...10
Dr. M, Morb. Basedowii.....15
Typhus No. 2.....20

/1093

Dr. St., Dr. Fl., Dr. Kfd.....25
Dr. S.....30

Some of these persons were tested again after three months, using the same serum. The results were the same.

With regard to the sera, we never observed agglutination in dilutions above 35.

Ascoli and Eisenberg also found the usual limit of effectiveness at a 20-30 dilution; thus, the intensity of the isoagglutinins has about the same sphere as the agglutinins for the typhus bacillus, occurring in healthy persons.

As for chemical composition, the agglutinins appear to belong to the globulins. Widal and Siccard, as well as Pick [13] showed for bacteria agglutinins, and Landsteiner showed for (hetero-) hemagglutinins that they are precipitated from the serum by globulin-precipitating substances. We can attest the same proof for isoagglutinins: if a serum is mixed with an equal quantity of saturated ammonium sulfate solution and then filtered, the filtrate has no hemagglutinating power, whereas the residue on the filter, if dissolved in physiological NaCl solution will agglutinate corresponding to the type of serum*.

By heating for one-half hour to 56°C, the isoagglutinins are not inactivated as is the case with hemolysins, however, we must emphasize that their effectiveness is thereby considerably impaired. Storage for several months in sealed glass tubes does not influence the effectiveness.

Concerning the relation of the isohemagglutinins to the specific bacteria agglutinins, Ascoli stressed that he found no parallelism between the two. We

---

*When testing a large series of bloods for the determination of the blood type, one could simply use three standard solutions of the agglutinating substance of the different serum types, by redissolving the ammonium sulfate precipitates in physiological NaCl solution.

tested this question in the following manner: we determined the agglutinating power of the blood of several typhus convalescents for the typhus bacillus and then we deprived the serum of its agglutinating capacity by mixing it with blood cells. The recently conducted test according to Gruber and Widal, showed no difference in the agglutinating power for the bacilli.

We will now turn to a discussion of circumstances we observed during the study of the blood of very young individuals.

For the possibility to conduct these investigations, we owe the kind permission of Professors Piskatschek and Monti, as well as of Dr. Riether, and would like to thank these gentlemen at this point.

Behavior of the blood of neonates and children under six months of age.

TABLE III.

COMPARISON OF THE BLOOD OF THE MOTHER AND OF THE CHILD (PLACENTA)

No.	Name of mother and child	Serum of the mother acting on a suspension of blood from:				Serum of the child (placenta) acting on a suspension of blood from:			
		Group A	Group B	Group C	type of the mother's blood	Group A	Group B	Group C	type of the child's blood
1	Wein.	+	0	0	B	+	0	0	B
2	Mar.	+ (weak)	+ (clear)	0	C	0	+	0	A
3	Gresb.	+	+	0	C	+	+	0	C
4	Bres.	+	+	0	C	+	0	0	B
5	Burk.	0	+	0	A	0	+	0	A
6	Solta	not investigated				+	0	0	B
7	--					0	0	0	-
8	--					0	0	0	-

Thus the blood of 2 out of 8 neonates had no agglutinins, and in 6 cases, the agglutinin behaved identically to the agglutinin of adults. However, in most cases the intensity of agglutination was decidedly lower than in the blood of adults, and in cases 1 and 2 it only occurred after 10 minutes. The blood cells of the children corresponded characteristically to the serum, inasmuch as in cases 1, 3 and 5, they were not agglutinated by the corresponding mother-serum, whereas in case 4 agglutination was observed (corresponding to the type). Case 2 seemed to behave atypically, inasmuch as the child-blood (type A) was not agglutinated by the mother-serum (type C).

A second study of children aged 7 days to 4 months, revealed atypical behavior in most of the cases.

TABLE IV.

11 CHILDREN (AGED 7 DAYS TO 4 MONTHS). EFFECT OF THE CHILD-SERUM ON BLOOD SUSPENSIONS OF THE THREE GROUPS.

No.	Sex	Age	Blocd suspension of type:			Type of child-serum
			A	B	C	
1	Male	4 mos. 10 days	0	0	0	-
2	Male	4 mos. 4 days	+	0	0	B
3	Female	3 mos. 5 days	0	0	0	-
4	Male	3 mos.	0	0	0	-
5	Female	3 mos.	+	0	0	B
6	Male	2 mos. 25 days	0	0	0	-
7	Female	2 mos. 5 days	+	+	0	C
8	Female	1 mon. 21 days	0	0	0	-
9	Male	1 mon.	0	0	0	-
10	Female	16 days	+	+	0	C
11	Male	7 days	0	0	0	-

Of these 11 children, only 4 had agglutinins in their serum.

In order to determine the behavior of the erythrocytes, the blood cell suspensions of all children were mixed with different sera from children and adults. The results are shown in Table V.

If we summarize the results evident from Tables III, IV, and V, we can distinguish four groups in the children studied.

TABLE V.

Serum:		Blood suspension from child no.										
		1	2	3	4	5	6	7	8	9	10	11
Group A	1. Dr. Stur. 2. Dr. Flechs.	0	0	0	+	0	0	0	0	0	0	0
B	1. Dr. Kl. 2. Chorea 3. Child No. 2 (Table IV)	+	0	+	+	0	0	0	+	+	0	0
C	1. Dr. Dec. 2. Ca. ventr. 3. Child No. 6 (Table IV) 4. Child No. 9 (Table IV)	+	0	+	+	0	0	0	+	+	0	0
Sera w/o type	Child No. 3, 4, 5 and 7 (Table IV)	0	0	0	0	0	0	0	0	0	0	0

1. Blood (Table V, No. 4) whose serum had no agglutinating capacity at all, but whose blood cells were agglutinated by the sera of all three types. This child was identical with the 4 adults (see above) in whose serum we found

---

Note: In cases 7 and 8, from Table III, we studied only the behavior of the serum, but not of the blood cells; thus, we cannot say whether these sera belong to group 1 or 2.

no agglutinin.

2. Six cases in which the serum did not agglutinate any blood type, /1094 but whose blood cells showed the same regular behavior toward the various typical sera: the blood cells of numbers 1, 3, 8 and 9 of Table IV are agglutinated by all sera of type B and C; those of numbers 6 and 11 from Table IV are not agglutinated by any serum; the former would thus correspond to type A, the latter to type C.

3. Three cases in which the serum was of one of the simple types, but where the blood cells were of the double type. In number 2 from Table III, the serum belonged to group A, but the blood cells were not agglutinated by the mother-serum (C); in number 2 from Table IV, the serum agglutinated group A, i.e., is of the type B, but the blood cells are resistant to any serum and must therefore be considered to be of type C. The same is true for number 5, Table IV.

4. Finally, a series of cases whose blood could be grouped with the blood of older individuals, as regards the serum as well as the blood cells, with the only difference that the agglutinating power of the serum was usually much lower than in the case of adults.

Thus, we can see that in younger persons the circumstances are not quite as simple and clear as in adult. Of course we must first look to the developmental process which leads to stationary circumstances at a later time. If one would wish to follow this process on the basis of the four established groups, it would seem as if the original state of each blood variety would be that of group 1, where no agglutinin is found in the serum and where the blood cells are influenced by any serum which contains agglutinin, and thus do not have any specific resistance. (As we have shown, this state can also be found in rare

cases of adults). In most individuals, since a typical resistance of the red blood cells to certain sera can even be found at the time of birth or in the first years of life even though the serum contains no agglutinin, it would seem to follow that the occurrence of the agglutinins is preceded by a differentiation of the erythrocytes, which would be the primary process (group 2). The last process would be the development of the agglutinins in the serum (groups 3 and 4). However, there are many objections to this assumption, since, on the one hand, the assumed primary differentiation consists of a specific indifference to certain agglutinins and it seems contradictory that the occurrence of this characteristic should precede the formation of the agglutinating substance. On the other hand, this assumption does not explain why the erythrocytes are resistant to the agglutinin of their own blood, while being sensitive to that of a different blood. We will attempt a possible explanation, which does justice to these objections. As mentioned before, group 1 probably corresponds to the original fetal state of each blood. At some later date, sometimes before birth, sometimes in the first years of life, a substance with agglutinating power is formed in the serum (in a similar way to the formation of bacteria agglutinins of unknown origin). It must now be assumed that this substance will first of all affect the erythrocytes of the same person. Since the formation of a substance of this kind probably does not occur suddenly, one can imagine that the agglutinating blood cells gradually develop to a state of increased resistance or immunity to this agglutinin. Similar experiences were noted with hemolytic cases. Glay and Cannes [14], Kossel [15] and Tschistovitch [16] found that by continued injection of an initially strong hemolytic eel serum, one not only causes the formation of an antibody in the serum of rabbits, but also the erythrocytes themselves become highly resistant, even completely

immune (Glay and Cannes) to hemolysin. Since, in the case of such an immunization, one must assume a certain consumption of the effective substance, in our case of the agglutinin (possibly a binding to the erythrocytes), it would be understandable at this stage of the process, if one could even observe a certain resistance of the erythrocytes to agglutinin, whereas the serum would not yet have developed this resistance. All the produced agglutinin would be bound to the erythrocytes, or would be consumed for the purpose of their immunization. If the immunization once progresses to the point where the agglutinating substance finds few or no points of attack on the red blood cells, it will freely occur in the serum and thus determine the type of the serum. The occurrence of the double type could be understood in terms of the occurrence of both agglutinating substances in the serum (earlier in our report we expressed the opinion that we are dealing with one substance, subject to chemical modification, possibly isomerism). The blood cells are immunized to both of these substances. The two agglutinins do not have to occur at the same time, or possibly one could occur in greater quantities than the other, so that at a certain point the blood cells would already be immune to both substances, whereas only one can be detected in the serum. In that case, the blood is of the double type, while the serum is of the simple type (Table III, No. 2; Table IV, No. 2 and No. 9).

We had the opportunity to re-examine 4 children of Table IV after 17 days. In numbers 4, 5 and 8, the result was the same; in number 2, the blood cells were now of type A (previously C) and the serum showed no agglutinin (previously type B). It would therefore appear that in the first months of life, one of the agglutinins (in this case type B) can undergo involution.

The question as to how the agglutinins enter the serum, we must, of course, leave unanswered.

We have already shown that pathological processes do not yield any explanation. The opinion of Ascoli and Eisenberg, viz, the phenomenon was caused by pathological blood decomposition, would have to be transferred to physiological blood decomposition, because of its occurrence in neonates.

However, this assumption is open to several objections, some of which have already been pointed out by Halban and Landsteiner.

Thus, corresponding to the double character of the agglutinating substance, one would have to assume a primary differentiation of the erythrocytes into 2 or 3 types.

Further, it would be hard to understand how instances are possible in which the agglutinins are completely lacking in an older person, and why isoagglutinins do not occur in any other species. We could not find any in dogs, guinea pigs or rabbits.

According to our above-mentioned assumption of the occurrence of autoimmunization of the erythrocytes through the isoagglutinins, we would, of course, have to admit that the detection of isoagglutinating substances in the blood of a species must depend on the presence of at least two such substances. Because, if in all individuals of a species only one and the same agglutinin is present, no serum could react with the blood of another representative of the same species (even if immunization of the erythrocytes is admitted), since the latter's blood cells are immunized to the same agglutinin. The proof of this could only be established by testing the blood of very young individuals with the serum of older ones.

Finally, it should be expected that the decomposition of cells would not

only be caused by agglutinins, but also by a hemolysin, with the same characteristic behavior as the former. However, this is not the case. As with Eisenberg, we could never observe a clear hemolytic effect for a serum during observation in suspended drops.

Therefore, we would like to take exception to the opinion that the isoagglutinins are formed by normal blood decomposition*. It seems much more likely to us, as it does to Halban, to relate these substances to other agglutinins (for various bacteria, as well as for blood cells of different species) which are normally found in the serum. We have already pointed out that their activity is about the same.

Let us mention that during the 4 months of observation, numerous reexaminations never revealed a change in the blood type.

The results of this study can be summarized as follows: /1095

1. The serum of the majority of healthy and sick persons, aged 6 months or more, contains isohemagglutinins. (Among the 155 persons examined, we found 4 exceptions = 2.5%).

2. The typical behavior of serum and blood cells, as described by Landsteiner, is found in healthy as well as sick individuals, both regularly and in the same manner.

3. The exceptions are merely marked by a complete lack of isoagglutinins and in the specific insensitivity of the erythrocytes.

4. Isoagglutination has no diagnostic significance.

5. In neonates and in children under 6 months of age, apparent deviations from the typical behavior can be observed, which can be explained by the

---

*Kraus and Ludwig [17] have reached the same conclusion through a series of experiments recently published (Wiener klin. Wochenschr. No. 15).

assumption that the agglutinins occur primarily in the serum, and secondarily cause an alteration (immunization) of the red blood cells.

6. Physiological and pathological blood decomposition is probably not the cause for the occurrence of isoagglutinin.

7. Rouleau formation has nothing to do with the effect of the isoagglutinins.

Vienna, April, 1902.

REFERENCES

1. Landsteiner, K.: The antifermentative, lytic and agglutinating effects of the blood serum and of the lymph. Centralbl. f. Bakt. Vol. XXVII, p. 361.
2. Shattock: Journ. of Path. and Bact. 1900.
3. Donath: Wiener klin. Wochenschr. p. 497, 1900.
4. Ascoli: Münch. med. Wochenschr. p. 1239, 1901.
5. Camus and Pagniez: Comptes Rendus p. 1020, 1901.
6. Eisenberg: Wiener klin. Wochenschr. p. 1021, 1901.
7. Lo Monaco and Panichi: Rendic. delle R. Accad. dei Lincei, Dec. 16, 1900; Ref. in Centralbl. f. allg. Path. u. path. Anat. 12: 338, 1901.
8. Grixoni: Faz. degli osped. No. 57, 1901; Ref. in Centralbl. f. inn. Med. p. 934, 1901.
9. Grünbaum: British Med. Journ. p. 1089, 1900.
10. Halban: Wiener klin. Wochenschr. No. 24, 1900.
11. Landsteiner: Wiener klin. Wochenschr. 46: 1132, 1901.
12. Kraus and Clairmont: Wiener klin. Wochenschr. 1900; Ibid. No. 13, 1901.
13. Pick: Hofmeisters Beitr. z. chem. Phys. u. Path. 1901 and 1902.
14. Glay and Cannes: Semaine Médicale No. 7, 1898.
15. Kossel: Berliner klin. Wochenschr. No. 7, 1898.
16. Tschistovitch: Annales de l'Institut Pasteur No. 5, 1899.
17. Kraus and Ludwig: Wiener klin. Wochenschr. No. 15, 1902.

ON THE GROUP-SPECIFIC STRUCTURES OF THE BLOOD

E. von Dungern and L. Hirszfeld

Translation of "Über gruppenspezifische Strukturen des Blutes". Zeitschrift für Immunitätsforschung und Experimentelle Therapie 8: 526-562, 1911.

U. S. ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX KENTUCKY 40121

BLANK PAGE

ON THE GROUP-SPECIFIC STRUCTURES OF THE BLOOD. III.

Prof. v. Dungern and Dr. Hirschfeld

1. STUDIES OF HUMAN BLOOD KINDS WITH HUMAN AGGLUTININS */526

As has been mentioned in the preceding communication, human blood corpuscles can be readily differentiated into four groups, with the aid of normal isoagglutinins, depending on whether they do or do not possess structures A or B, thus: A and B; A and non-B; non-A and B; non-A and non-B (O).

Moss too, who recently reported his results on 1600 studies (Bull. of the Johns Hopkins Hospital, March 1910, Baltimore), was able to divide all of the blood samples into four groups, which coincide with ours. Actually he believes, in contrast to Landsteiner's statement, that three separate components must be assumed in order to explain the four groups; this view is however incorrect, and is explainable by the fact that he has not taken into consideration the joint occurrence of A and B. His group I coincides with group AB, his group II is definitely A, his group III is therefore B and his group IV is O. It is highly remarkable that the numerical ratios found by him in Baltimore practically completely coincide with ours, which had been obtained in Germany.

	A	A without B	B	B without A	A and B	Neither A nor B
Heidelberg	53%	47%	16%	11%	5.7%	36%
Baltimore	50%	40%	17%	7%	10%	43%

Genetic investigations have shown that the tendencies to A and and to /527 B constitute genetic entities that are independent of each other, whereas A and

*/Numbers in the margin indicate pagination of original foreign text.

non-A, B and non-B, influence each other in accordance with Mendelian laws. The presence of A and B can usually be very clearly demonstrated. Agglutinins are either highly potent or are entirely absent; and only in individual cases are the blood corpuscles weakly agglutinized. In addition to these quantitative differences, there are also discernible qualitative ones. Thus it will be found that the sera of persons whose blood contains B and non-A, and which therefore act in accordance with Landsteiner's rule, upon A, behave differently from each other. In our own experiments we used mainly three sera (Deetjen, Wernicke, Oberin). Two of these acted on a greater number of human blood varieties than the third did (Oberin), although the number of persons whose blood would react with two of the sera only was relatively small. In one such case the corresponding serum was also tested; it was found not to contain alpha. We designate the structure which does not react with the Oberin serum but definitely does with the serum of both of the other men of the B group, by the term "little A". Still further differences were found which corresponded to Langer's observations (Zeitschr. f. Heilkunde, 1903), when we absorbed a given serum which contained alpha with a certain A-containing type of blood. Something yet remained, which still was capable of acting on a few of the types of blood which were influenceable before absorption.

Sera	A						B		AB	O			
	Marie	Otto	Ludwig	Schw. Anna	Hanna	Johann	Oberin	Wernicke	Anna	Kalchschm.	Karl	Amalie	Hirschfeld
Oberin (B) - Marie (A)	0	±	±	±	±	±	0	0	±	0	0	0	0
Oberin (B) - Ludwig (A)	0	.	0	.	.	0	.	.	.	.	.	.	

We thus showed that here are types of blood which could be agglutinated by the Oberin serum even after absorption with Marie blood.

We were able, on the bases of these sera, to distinguish between three /528 kinds of blood in group A.

1. Those kinds of blood which could be agglutinized with the Oberin serum even after absorption with Marie blood.
2. Those kinds of blood which would be agglutinated by the Oberin serum, but no longer so after absorption of this serum with Marie blood.
3. Those that could not be agglutinated by the Oberin serum at all, but could be agglutinated by the Deetjen, Wernicke, and other sera.

Absorption of the Wernicke serum with Marie blood did not yield any discernible differences. The variations were recognizable, although we undertook only a small number of absorptions with human serum. We conducted this examination twice and found that the same relations prevailed even a half year later.

STUDIES OF HUMAN BLOOD WITH ANIMAL AGGLUTININS

We were interested in finding still other specific components in the human blood, and for that reason experimented also with animal sera.[1] In general, animal sera agglutinated all kinds of human blood in the same manner. Only a few constitute an exception; most of them could therefore not be directly used for purposes of differentiation. It proved possible, however, with the aid of the absorption method, to almost always modify the animal sera in such a fashion that they would react only upon a fraction of the human blood varieties. We had undertaken a total of about 150 absorptions and conducted tests on a certain number of human kinds of blood. To the extent that it was possible to

[1] Preliminary communications: Münch. med. Wschr. 1910, nos. 6 and 14.

do so, the same persons were employed for the investigations. The serum was heated for one-half hour at 60° in order to eliminate the complement. The blood corpuscles that were used for absorption were separated from the serum, repeatedly washed with physiologic saline solution, condensed by rapid centrifugation, and added to the animal serum in equal parts. The blood remained in contact with the serum for at least one hour. It was then centrifugated /529 off, and the serum tested for its effectiveness upon the blood kinds being used for absorption. If agglutination still occurred, then absorption would be repeated; in general, however, one treatment would suffice. Only in the case of cat serum and immune sera was it necessary to repeat absorption several times. The blood corpuscles being used in this study were employed in an approximately 3% suspension, without removal of the serum. The blood from each person was suspended in a fluid which consisted of 5 parts of 0.85% saline solution and one part of 3.2% sodium citrate solution. The blood corpuscles being investigated were always removed and tested on the same day. The quantities of serum added were always equal to those of the blood-corpuscle suspension. Results were measured after about three hours, and rechecked after about 20 hours: ++ indicates agglutination into thick clumps which could not be broken up by agitation, + indicates total agglutination of the blood corpuscles into clumps which could be broken up into smaller bits, but not back into the individual blood corpuscles, while ± indicates agglutination of individual blood corpuscles into small piles. Very poor agglutinations were no longer considered. When experiments of the same kind were repeated slight differences would often be found. Yet considerable differences could not be abolished by repeated tests. The records presented below indicate the results of the experiments. The first column represents the sera used for absorption; thus, for example,

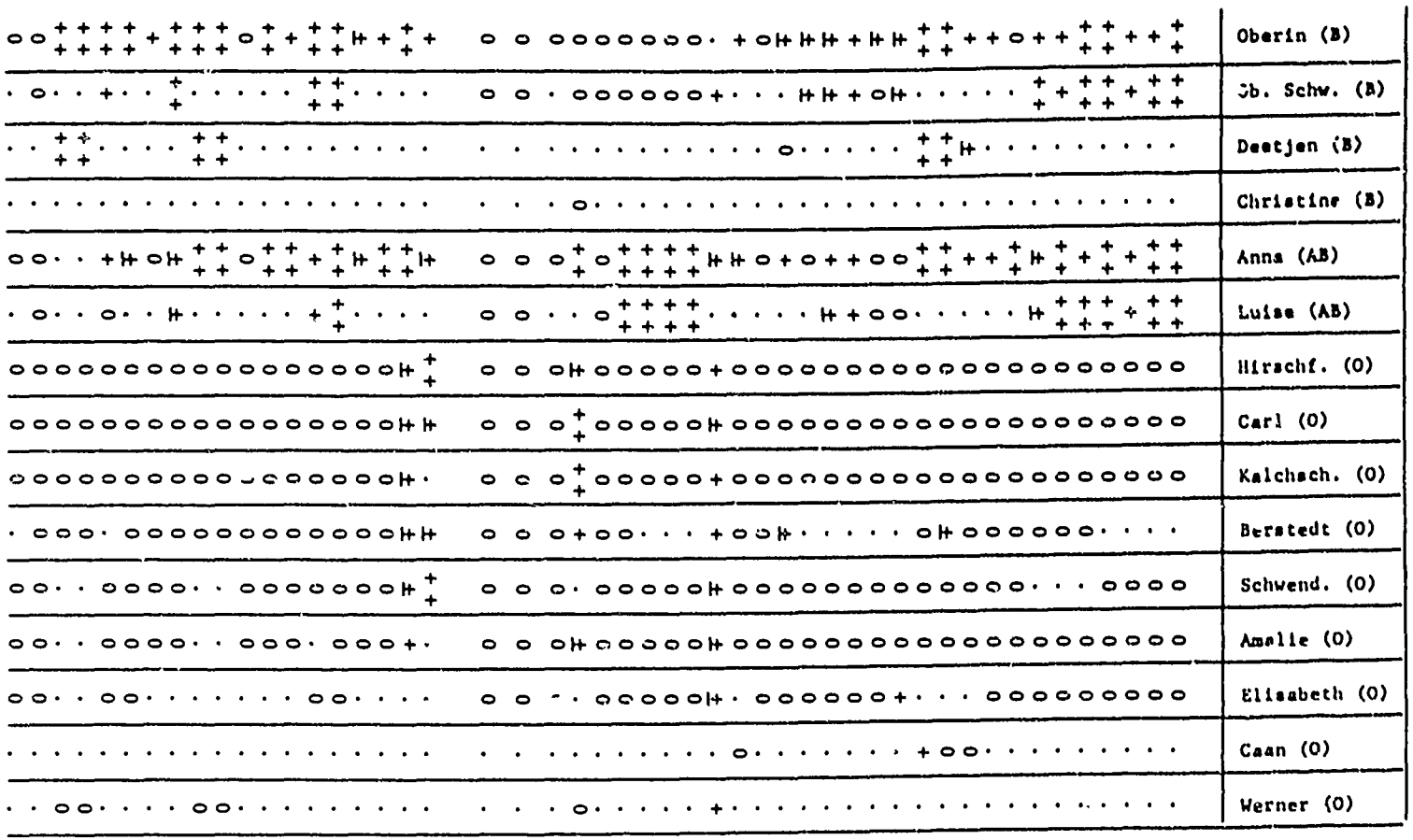
"Katz-Marie" (i.e., cat-Marie) indicates the serum of a cat which was absorbed with Marie's blood in the manner described. On top, are the blood kinds investigated, arranged so that the order of persons is: group 1 — A and non-B; 2 — B and non-A; 3 — A and B; 4 — non-A and non-B. Also, the absorbed sera are arranged in accordance with their absorption by the blood of the various groups. A symbol next to the blood used for absorption indicates the group to which it belongs.

The results show that when animal serums are used the same groupings /536 frequently arise as in the case of human sera. Thus the sera behave as though they contained alpha, or beta, or alpha and beta. In other cases there is a residual selective action on those blood kinds which contain neither A nor B, and finally, there are also to be found differences which are based on the variability within the individual groups characterized by the isoagglutinins. It may happen that blood with a component A or B will behave differently from other blood corpuscles of groups A or B, and will behave in the same way as do other kinds of blood which contain non-A or non-B.

The grouping in terms of A and B shows up particularly distinctly in the actions of the sera from cattle and rabbits. Thus bovine serum 1 was absorbed with the blood of Karl (O), Hirschfeld (O), Elisab... (O), Otto (A), Marie (A), Bochen (A), Wernicke (B), Oberin (B), Anna (A and B) and Luise (A and B). After absorption with a group O kind of blood the serum would agglutinate all kinds of blood which contained A or B, but not one that contained O. The kinds of blood which contained component B changed the serum in such a fashion that it would act only upon A, but would no longer do so on B. The blood corpuscles which contained both components (A and B) would capture everything, whereas following absorption with a group A blood the serum would react only with B

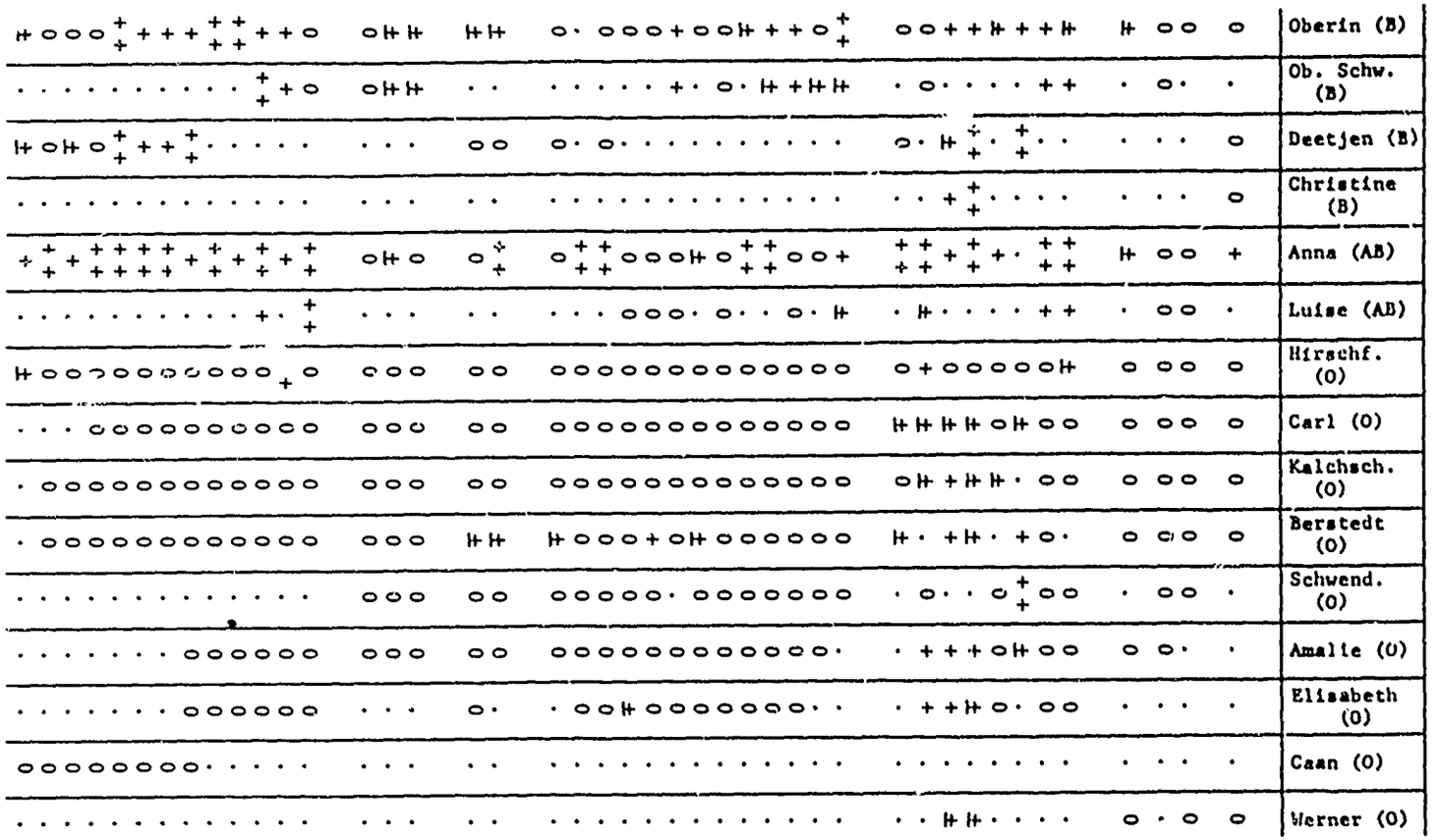
Reco da

Sera	Marie (A)	Ida (A)	Bochen (A)	Otto (A)	Hanna (A)	Johann (A)	Schw. Anna I (A)	Heizer (A)	Sch. Anna II (A)	Ludwig (A)	Wernicke (B)
Cattle:											
1 - Carl (O)	+ +	+ +	.	+ +	+ +	+ +	+ +	.	.	.	+ +
3 - Carl (O)	+	+	.	+ +	+	+ +	+ +	.	.	.	+ +
4 - Carl (O)	0	0	.	±	0	.	±	.	.	.	+ +
1 - Hirschfeld (O)	+	.	.	+	+	.	+ +	.	.	.	+
4 - Hirschfeld (O)	0	.	.	0	0	.	0	.	.	0	+
1 - Elisabeth (O)	+ +	.	+ +	+ +	+	.	+ +	±	.	+ +	±
4 - Elisabeth (O)	0	.	0	0	0	.	0	0	.	0	±
5 - Hirschfeld (O)	+ +	.	.	+ +	+ +	.	.	.	.	+ +	0
6 - Hirschfeld (O)	0	.	.	+	+	.	.	.	.	±	0
8 - Caan (O)	0	.	.	+	±	.	.	.	±	+	±
9 - Hirschfeld (O)	+	.	.	+ +	+ +	.	.	+ +	+ +	+ +	+ +
10 - Hirschfeld (O)	+	.	.	+ +	+ +	.	.	+ +	+ +	+ +	+ +
1 - Otto (A)	0	0	.	0	0	0	0	.	.	.	+
3 - Otto (A)	0	0	.	0	0	±	±	.	.	.	±
4 - Otto (A)	0	0	.	0	0	0	0	.	.	.	+
1 - Marie (A)	0	0	.	+	0	±	±	.	.	0	±
1 - Bochen (A)	0	.	0	0	0	0	0	.	.	0	0
5 - Ludwig (A)	0	.	.	±	+	.	.	.	.	0	0
7 - Marie (A)	0	.	0	0	0	.	.	.	.	0	0
8 - Marie (A)	0	.	+	0	0	.	.	.	.	±	0
11 - Heizer (A)	+	.	.	.	0	.	.	± 0	0	0	+ +
1 - Wernicke (B)	+	+	.	+ +	+ +	+ +	+ +	.	.	.	0
2 - Wernicke (B)	+	+	.	+ +	+ +	+ +	+ +	.	.	.	0
3 - Wernicke (B)	+	+	.	+ +	+ +	+ +	+ +	.	.	.	0
1 - Oberin (B)	+	.	.	+	+ +	+ +	.	.	.	.	0
4 - Oberin (B)	0	.	.	0	0	0	.	.	.	.	0
10 - Deetjen (B)	.	.	.	.	±	.	.	+ +	.	+ +	0
4 - Wernicke (B)	0	0	.	±	0	.	.	.	.	.	0
1) 4) - Anna (AB)	0	0	.	0	0	.	.	0	0	0	0
1 - Luise (AB)	0	.	0	0	0	.	.	0	0	0	0
Rabbit:											
2 not absorbed	.	.	±	.	.	.	.	.	.	.	+ +
6 not absorbed	+ +	+ +	+ +	.	.	.	.	.	.	.	+
7 - Carl (O)	+ +	+	+	.	.	.	.	.	.	.	.
8 - Carl (O)	0	0	0	.	.	.	.	.	.	.	.
- Hirschfeld (O)	±	.	.	±	0	.	+ +	.	.	.	+ +
20 - Elisabeth (O)	±	.	+	+	±	.	±	0	.	+	+ +
9 - Carl (O)	+	+	+	.	.	.	.	.	.	.	+
10 - Carl (O)	±	±	+	.	.	.	.	.	.	.	+ +
11 - Carl (O)	0	0	0	.	.	.	.	.	.	.	0
302 - Hirschfeld (O)	.	.	+ +	.	.	.	+ +	.	.	+ +	.
302 - Carl (O)	.	.	+ +	.	+ +	.	+ +	.	.	+ +	.
20 - Bochen (A)	0	.	0	0	0	.	0	0	.	0	+ +
10 - Ida (A)	0	0	±	.	.	.	.	.	.	.	+ +
10 - Otto (A)	0	0	.	0	0	.	.	.	.	.	+ +
10 - Marie (A)	0	0	.	0	0	0	0	.	.	.	+ +
360 - Bochen (A)	.	.	0	.	0	.	0	.	.	.	.
435 - Bochen (A)	.	.	0	.	0	.	0	.	.	.	.
13 - Oberin (B)	0	0	.	.	.	.	.	.	.	.	0
170 - Wernicke (B)	0	0	.	+	0	.	.	.	.	.	0

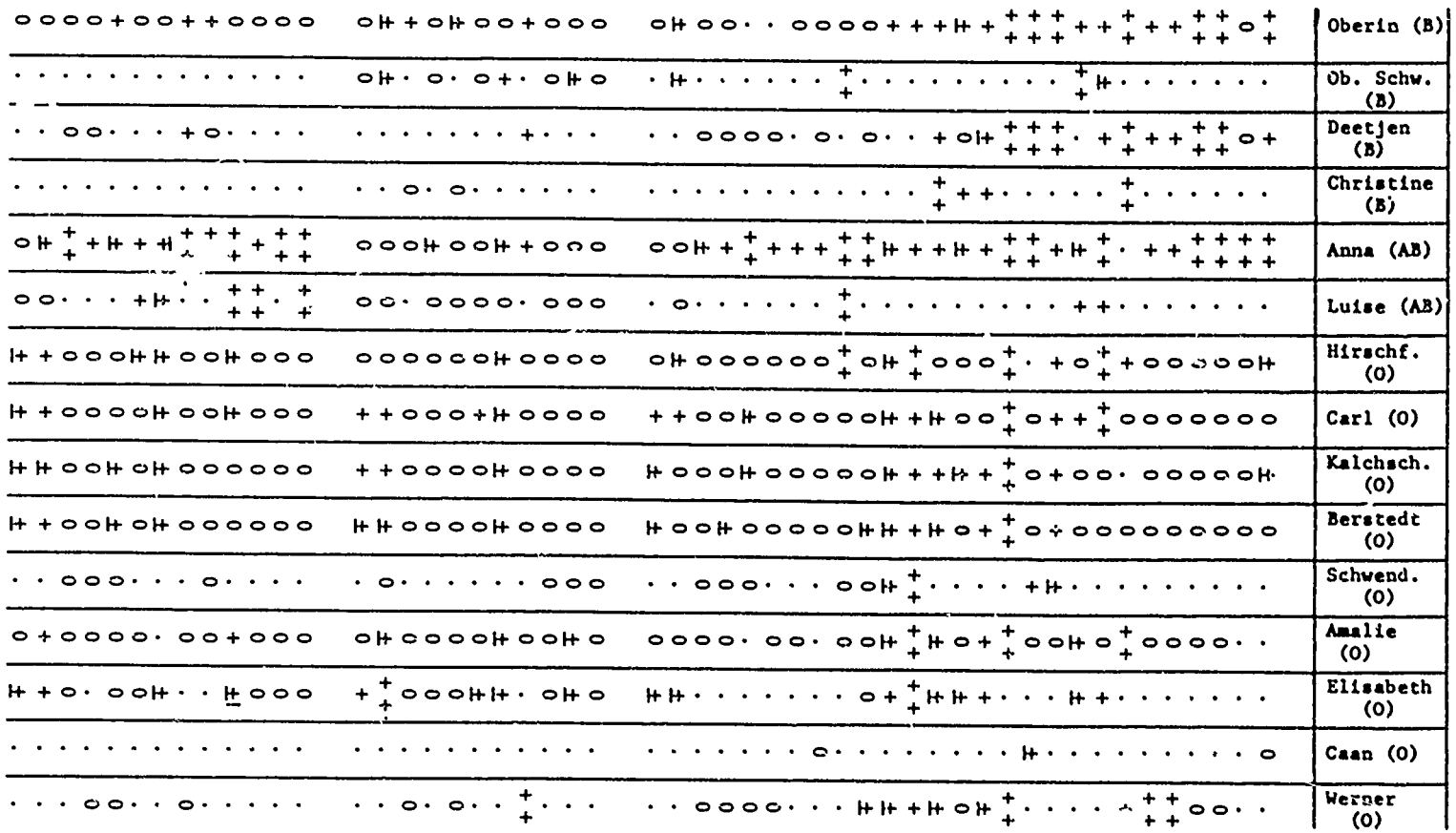

Oberin (B)
Ob. Schw. (B)
Deetjen (B)
Christine (B)
Anna (AB)
Luise (AB)
Hirschf. (O)
Carl (O)
Kalchsch. (O)
Berstedt (O)
Schwend. (O)
Amalie (O)
Elisabeth (O)
Caan (O)
Werner (O)

Sera	Marie (A)	Ida (A)	Bochen (A)	Otto (A)	Hanna (A)	Johann (A)	Schw. Anna I (A)	Heizer (A)	Sch. Anna II (A)	Ludwig (A)	Wernicke (B)
Rabbit:											
6 - Wernicke (B)	.	.	.	.	.	.	+ +	.	.	+ +	0
10 - Anna (AB)											
(7 Anna)	0	0	0	.	0	.	.	.	.	.	+
20 - Luise (AB)	0	.	0	0	0	.	0	0	.	0	0
Hammel-Hirschfeld (O)	.	.	+	.	+	.	+	.	.	+	.
Goat:											
1 - Carl (O)	+	±	.	+ +	+ +	+	+	.	.	.	+
2 - Carl (O)	+	.	.	+ +	+ +	+ +	+ +	.	.	.	+ +
jung[1] -Hirschfeld (O)	±	.	.	+	+	.	.	.	.	.	+
1 - Marie (A)	0	0	.	±	0	.	.	.	.	+	±
jung-Ludwig (A)	.	.	.	.	.	.	±	.	.	0	+ +
3 - Ludwig (A)	.	.	.	.	.	.	.	.	.	0	±
1 - Wernicke (B)	+	±	.	+ +	+	+ +	+ +	.	.	.	0
3 - Deetjen (B)	±	.	.	+	±	.	.	+ +	0	±	0
Dog:											
21 - Hirschfeld (G)	0	.	.	0	0	.	0	.	.	.	+
22 - Carl (O)	0	.	.	0	0	.	0	.	.	0	±
23 - Elisabeth (O)	0	.	0	±	0	.	0	0	.	0	0
13 - Carl (O)	+ +	.	.	+ +	+ +	.	.	.	.	+ +	±
- Carl (O)	+ +	.	.	+ +	+ +	.	.	.	.	+ +	±
27 - Marie (A)	0	.	.	0	0	0	0	.	.	0	±
21 - Marie (A)	0	.	.	0	0	0	0	.	.	0	+
23 - Bochen (A)	0	.	0	0	0	.	0	0	.	0	0
21 - Wernicke (B)	0	.	.	0	.	±	0	.	.	0	±
21 - Oberin (B)	0	.	.	0	.	0	±	.	.	0	0
13 - Wernicke (B)	+	.	.	+ +	+ +	.	.	.	.	+ +	0
13 - Deetjen (B)	+	.	.	+ +	+ +	.	+	+	.	+ +	0
21 - Anna (AB)	0	.	.	0	0	.	0	.	.	0	0
Monkey:											
- Hirschfeld (O)	+ +	.	.	+ +	+ +	.	.	.	.	+ +	0
- Ludwig (A)	±	.	.	0	0	.	.	.	.	0	0
Chicken:											
1 - Marie (A)	0	.	.	0	0	.	.	.	.	.	±
2 - Marie (A)	0	.	.	0	0	.	.	.	.	.	±
1 - Wernicke (B)	0	.	.	0	0	.	.	.	.	.	0
Cat:											
1 - Hirschfeld (O)	+ +	.	.	+ +	+ +	.	+ +	.	.	+ +	0
1 - Carl (O)	+ +	.	.	+ +	+	.	+	.	.	+ +	+ +
20 - Elisabeth (O)	±	.	+	+ +	±	.	+	.	.	+ +	+ +
2 - Hirschfeld (O)	0	.	.	+ +	+ +	.	.	.	.	+ +	+ +
2 - Carl (O)	+ +	.	.	+ +	+ +	.	.	.	.	+ +	+ +
3 - Caan (O)	0	.	.	+ +	±	.	.	.	+	+ +	+ +
4 - Hirschfeld (O)	±	.	.	+ +	+ +	.	.	+ +	+ +	+ +	+
5 - Hirschfeld (O)	+	.	.	+ +	+ +	.	.	+ +	.	+ +	±
6 - Hirschfeld (O)	±	.	.	+ +	+ +	.	.	+ +	+ +	+ +	+ +
7 - Hirschfeld (O)	+	.	.	+ +	+ +	.	.	+ +	+ +	+ +	0
5 - Carl (O)	0	.	.	+	±	.	.	+ +	0	+ +	±
7 - Carl (O)	0	.	.	+	±	.	.	+	±	+	0
4 - Carl (O)	±	.	.	+	±	.	.	+	0	+	±

[1]Pretreated with human blood.


Oberin (B)
Ob. Schw. (B)
Deetjen (B)
Christine (B)
Anna (AB)
Luise (AB)
Hirschf. (O)
Carl (O)
Kalchsch. (O)
Berstedt (O)
Schwend. (O)
Amalie (O)
Elisabeth (O)
Caan (O)
Werner (O)

Sera	Marie (A)	Ida (A)	Bochen (A)	Otto (A)	Hanna (A)	Johann (A)	Schw. Anna I (A)	Heizer (A)	Sch. Anna II (A)	Ludwig (A)	Wernicke (B)
Cat:											
6 - Carl (O)	+	.	.	+	+ +	.	.	+ +	±	+ +	+
1 - Berstedt (O)	0	.	.	0	+	.	.	+ +	.	±	0
10 & 13 - Hirschf. (O)	.	.	+ +	+ +	+ +	.	.	.	.	+ +	.
8 - Hirschfeld (O)	.	.	+ +	+ +	+ +	.	.	.	.	+ +	.
9 - Hirschfeld (O)	.	.	+ +	+ +	+ +	.	.	.	.	+ +	.
10 & 13 - Carl (O)	.	.	+ +	+ +	+ +	.	.	.	.	+ +	.
9 - Carl (O)	.	.	+	+ +	.	.	.	.	.	+ +	.
1 - Ludwig (A)	+	.	.	+	0	.	0	.	.	0	.
20 - Bochen (A)	±	.	0	±	0	.	0	0	.	0	+ +
2 - Ludwig (A)	+	.	.	+	±	.	.	.	.	0	+ +
2 - Marie (A)	0	.	.	+ +	±	.	.	.	.	+ +	+ +
12 - Bochen (A)	.	.	0	+ +	±	.	.	.	.	±	.
30 - Ludwig (A)	.	.	.	.	.		.	.	.	0	+ +
8 - Ludwig (A)	.	.	.	.	.	.	0	.	.	0	±
10 & 13 - Ludwig (A)	.	.	.	.	.	.	0	.	.	0	±
14 - Heizer (A)	.	.	.	.	±	.	.	0	.	0	+ +
15 - Heizer (A)	.	.	.	.	.	.	0	0	.	.	+ +
2 - Wernicke (B)	+ +	.	.	+ +	+ +	.	.	.	.	+ +	0
1 - Oberin (B)	+ +	.	.	+ +	+ +	.	+ +	.	.	+ +	+
7 - Deetjen (B)	±	.	.	+	±	.	0	+	.	+	0
6 - Deetjen (B)	±	.	.	+	±	.	0	±	.	0	0
6 - Christine (B)	.	.	.	.	+ +	.	.	.	+ +	+	0
12 - Deetjen (B)	.	.	.	.	.	+	+ +	.	±	+ +	0
12 - Oberin (B)	.	.	.	.	+	.	+ +	.	.	+ +	0
9 - Oberin (B)	.	.	.	.	0	.	+	.	.	±	0
1 - Anna (AB)	±	.	.	±	0	.	0	.	.	0	0
20 - Luise (AB)	±	.	0	0	0	.	.	.	.	.	0
Horse:											
1 - Hirschfeld (O)	0	.	.	+	0	.	0	.	.	0	0
1 - Carl (O)	0	.	.	0	0	.	±	.	.	0	±
1 - Elisabeth (O)	0	.	.	±	0	.	0	.	.	±	0
2 - Hirschfeld (O)	.	.	+ +	.	+ +	.	+ +	.	.	+ +	+ +
1 - Ludwig (A)	±	.	.	0	0	.	±	.	.	0	±
1 - Bochen (A)	0	.	0	0	0	.	0	.	.	0	0
2 - Heizer (A)	.	.	.	.	0	.	0	.	.	.	±
1 - Oberin (B)	0	.	.	±	.	.	0	.	.	.	0
2 - Wernicke (B)	.	.	.	.	.	.	+	.	.	+	0
1 - Anna (AB)	0	.	.	±	0	.	0	.	.	0	0
1 - Luise (AB)	+	.	0	0	0	.	0	0	.	(	0
Pig:											
1 - Hirschfeld (O)	±	.	+ +	+	+ +	.	+ +	+	.	±	±
2 & 3 - Hirschf. (O)	±	.	+ +	+	+ +	.	+ +	+ +	.	±	±
1 - Elisabeth (O)	±	.	+ +	+	+ +	.	+ +	+	.	+	±
2 - Elisabeth (O)	+	.	+ +	+	+ +	.	+ +	+ +	.	+	±
2 - Carl	+	.	.	+ +	+	.	.	.	.	+ +	±
5 - Hirschfeld	.	.	+ +	.	+ +	.	+ +	.	.	.	
1 - Bochen (A)	±	.	±1	0	0	.	0	0	.	±	(
2 - Bochen (A)	0	.	0	0	0	.	0	0	.	0	0
5 - Heizer (A)	.	.	.	.	0	.	.	.	.	.	+
4 - Oberin (B)	.	.	.	.	±	.	+	.	.	±	0
2 - Wernicke (B)	+	.	.	+ +	+ +	.	.	.	.	+	0
3 - Luise (AB)	±	.	+	0	0	.	0	0	.	±	0
1 - Anna (AB)	0	.	0	0	0	.	0	0	.	0	0


Oberin (B)
Ob. Schw. (B)
Deetjen (B)
Christine (B)
Anna (AB)
Luise (AB)
Hirschf. (O)
Carl (O)
Kalchsch. (O)
Berstedt (O)
Schwend. (O)
Amalie (O)
Elisabeth (O)
Caan (O)
Werner (O)

blood, although here too the action was weaker than after absorption with Karl and Hirschfeld blood. (The deviation for the group B blood perhaps is due to a spontaneous weakening of the agglutinin, since the serum was investigated later.) There are sporadic differences in the action on the various varieties of group A blood. The Bochen and the Otto bloods would capture the agglutinin for all blood varieties of the A group; the Marie blood, on the other hand, did not do so, sufficient agglutinin being left over for one-half of the investigated blood varieties of group A. Absorption with the Otto blood is further distinguished by the fact that it alone left behind sufficient agglutinin for one blood variety from group O (Elisabeth). The bovine sera 2 and 3 acted, following absorption with Wernicke blood (B), exclusively on the varieties of /537 blood containing A. The serum of cow 4 had a relationship primarily to the blood corpuscles of the B group and in addition to a slight extent to certain individual ones in the A group. It is remarkable that these same varieties of blood were still influenceable by Marie blood following absorption of bovine serum 1. Following absorption, bovine serum 5 exhibited an effect on all blood kinds in group A, but not on the members of group B. Absorption with Ludwig blood (A) removed the agglutinin for Marie blood, but on the contrary left behind that of Otto and Hanna. Furthermore, there still was present one agglutinin for the group O Berstedt blood, and the group B Oberin blood. Bovine serum 6 is particularly interesting: following absorption with the Hirschfeld blood it still possessed agglutinins for the Otto and Hanna bloods, but not for the Marie blood, and it separates in a similar fashion with respect to the varieties of group B blood, in the sense that it agglutinated only the Oberin blood, but not the Wernicke blood. The bovine serum 7 did not work at all after absorption with Marie blood (A). It therefore contained no agglutinins for the

B group. Bovine serum 8 behaved similarly to bovine serum 6. The sera of cattle 9 and 10 acted following absorption with Hirschfeld blood (C) primarily on varieties of blood from groups A and B. Bovine serum 10 behaved similarly following absorption with Hirschfeld blood. Of the kinds of blood in the C group, only the Caan blood was agglutinated. Peculiar behavior was noticed after treatment with Deetjen blood, powerful agglutinins being present for almost all of the kinds of the group O blood under investigation, while there were none for the investigated members of the B group. There were individual quantitative differences both for group O and for group A. Bovine serum 11, after absorption with Heizer (A) blood, shows similar behavior with respect to the kinds of blood of the O group, only the Werner blood being agglutinated in this case, which is not the case for bovine serum 10-Deetjen. Marie blood also forms an exception in this respect since it still is agglutinated despite absorption with a blood from the A group. In this case the blood varieties /538 of the B group were agglutinated. Thus it becomes possible, even with these few experiments, to undertake quite a large number of differentiations. In addition to the chief actions on groups A and B, we can also state that there are yet four other different agglutination actions on the blood of the O group: (1) Bovine serum 10-Hirschfeld agglutinated only Caan blood; (2) bovine serum 1-Otto agglutinated only Elisabeth blood; (3) bovine serum 10-Deetjen acted primarily on Carl, Kalchschmidt and Berstedt bloods but not on Werner blood; (4) bovine serum 11-Heizer acted on Hirschfeld, Berstedt, Kalchschmidt and Werner bloods. Furthermore sera 5 made it possible to discern various groupings within group A and two different ones within group B, consisting of: (1) no action on Marie blood, but action on Otto and Hanna bloods (bovine 6-Hirschfeld); (2) no action on Marie and Hanna bloods but action on Otto blood (bovine

1-Marie); (3) no action on Marie, Otto, Hanna bloods, but action on Bochen blood (bovine 8-Marie); (4) no action on Hanna blood, but action on Marie blood (bovine 11-Heizer); (5) action on all studied varieties of group A (the majority); (6) no action on Wernicke blood (B) but action on Oberin blood (B) (bovine 6-Hirschfeld, bovine 8-Marie); (7) on all investigated varieties of B-blood (the majority).

The agglutination action of the rabbit sera corresponds even better to grouping by A and B. Not a single blood of the non-A/non-B (O) bloods was agglutinated by any of the rabbit sera treated with human blood. On the other hand, these sera had an effect on blood varieties in the groups A and B. Yet here too there were great differences, most sera failing to agglutinate all blood varieties of group A, even when absorption had been performed with a blood from the non-A group. It was therefore possible, with the aid of these sera, to differentiate between different kinds of blood from group A. Thus, for example, rabbit serum 20-Elisabeth agglutinated strongly the Bochen, Otto and Ludwig bloods, but did not agglutinate Heizer blood; rabbit 170-Wernicke blood strongly agglutinated Otto blood but not Marie, Ida or Hanna blood; rabbit-Hirschfeld blood did not agglutinate Hanna blood, but agglutinated Sister-Anna I very strongly. The actions of the sera on the investigated group B /539 bloods were, generally speaking, uniform. Absorption with a blood from this group removed all of the agglutinin for all of the blood varieties in the B group, yet here too occasional differences could be noted. Rabbit 10-Anna, rabbit 7-Anna did agglutinate Wernicke blood, but not Oberin blood; rabbit 10-Marie affected only Anna blood, but not Luise blood. It is also remarkable that rabbit 20-Bochen agglutinated Wernicke, Oberin, Oberin-Sister, and Hirschfeld more powerfully than Anna and Luise bloods, which contained some A along

with their B.

The porcine sera contained above all agglutinins for the blood varieties in the A group; Marie blood being more weakly agglutinated. In addition, some of the sera possessed agglutinins for blood kinds belonging to group O. Of the blood varieties of group B, at times only Wernicke blood would be agglutinated, and at times Oberin blood as well. It is uncertain whether the porcine sera contained any agglutinin at all which will react with constituent B. This cannot be assumed as true for porcine serum 1, since following absorption with Bochen (A) blood, there remained no agglutinin for the B group.

The goat sera contained agglutinins for bloods from the A group, the B group, and in addition occasionally for some of the members of the O group. Different absorptions of the same serum with A blood or B blood yielded individual differences for bloods from the O group.

The equine serums did not exhibit a clear grouping by A and B. Different relations were found in each individual case; e.g., horse 1-Hirschfeld agglutinated Otto blood exclusively; horse 2-Hirschfeld agglutinated all kinds of blood from the A and B groups, while among the O group bloods it agglutinated only Werner blood; horse 1-Ludwig did not act upon Oberin blood, but did act on the Oberin-Sister blood; horse 1-Oberin affected neither the Oberin blood nor the Oberin-Sister blood; horse 2-Wernicke agglutinated Oberin blood, but not Wernicke or Christine blood (all in group B); horse 1-Anna and horse 1-Luise contain agglutinin for some of the varieties of the O group (Karl, Kalchschmidt, Elisabeth), but not for the Hirschfeld blood (group O); horse 1-Oberin, /540
horse 2-Wernicke, horse 2-Heizer possessed no agglutinin at all for the varieties of O group blood; horse 1-Bochen agglutinated Karl blood, but not the other kinds of O group blood. It is particularly striking that following ab-

sorption with Oberin blood there remained behind no agglutinin for Oberin-Sister blood, while when absorption is performed with Ludwig blood it was found that sufficient agglutinin remained for Oberin-Sister blood even after all of the agglutinin for the Oberin blood was removed. This phenomenon, which we encountered quite frequently, will be discussed later.

The A and B groupings did manifest themselves with canine sera. Some of the dogs do, others do not, contain A-agglutinin. The differences between the Wernicke and Oberin bloods were distinct, but actually differed depending on the serum and on which kind of blood was used for absorption. What is remarkable is that certain kinds of group O blood do cause this kind of absorption, whereas with other bloods of the group O agglutinins for both varieties of B group blood remain behind. It can therefore be stated that certain kinds of blood from the O group behave during absorption in the same way as certain kinds of blood from the B group. In general, the blood kinds which belong to the O group are not influenced by the absorbed canine sera. Dog serum 21 agglutinated the Berstedt blood but only after absorption with certain kinds of blood.

The A and B groupings are least pronounced in the tests with the feline sera. Actually, here too it was found that many sera act preferentially on the kinds of blood that are influenceable by human isoagglutinins; but in other instances this is by no means the case. Thus cat serum 1-Hirschfeld does not act on the blood varieties of the B group, although the Hirschfeld blood belongs to the O group. The same serum absorbed with Karl Blood (O group), does ag- /541
glutinate these kinds of blood and in addition the Hirschfeld blood. It may therefore be expected on the basis of this experiment that the Hirschfeld blood is identical to the blood varieties of the B group with respect to the agglu-

tinin of this serum. The absorption of this feline serum with Oberin blood does, however, leave behind a powerful agglutinin for the Hirschfeld blood (O) as well as for the Oberin-Sister (B) and the Wernicke (B) blood, although the agglutinin for the Oberin blood proper had been absorbed. The Anna blood, which contained blood B in addition to A, on the contrary almost completely absorbs agglutinin for Hirschfeld (O) blood, Wernicke (B) blood, Oberin (B) blood and Oberin-Sister (B) blood, and after this absorption there was found an agglutinin for the Karl blood which however was not at all influenced by the same serum following absorption with other kinds of blood. It will therefore be seen that almost every absorption forms new relations.

The investigated chick sera appear to possess a weak agglutinin for human blood of the B group.

The sera of the five studied Rhesus monkeys behaved in general as though they contained alpha only. The serum of a particular monkey had already proven itself to be specifically active even before absorption with human blood. This serum also agglutinated individual varieties of blood from the O group.

We investigated with particular interest the sera of two chimpanzees that had been sent to us by Professor Metchnikoff. One of these had a specific action even before absorption on individual varieties of human blood, and in fact on all varieties of group B blood and to a lesser extent on individual kinds of group A blo[illegible]s the bloods of the O group remained unaffected. The other serum agglu[illegible]ted all human kinds of blood to a very large extent, and after absorption with a blood from the O group only an agglutinin for the kinds of blood in the B group could be found.

Records

Sera	Str. (A)	R. (A)	M. (A)	L. (A)	C. (A)	N. (B)	W. (B)	P. (O)	M. (O)	S. (O)
	1	2	3	4	5	6	7	8	9	10
Monkey:										
I - Bl. (O)	+ +	+ +	+ +	+ +	+ +	0	0	0	0	0
II - Bl. (O)	+ +	+	+ +	+ +	+ +	0	0	0	0	0
III - Bl. (O)	hem.*	+	+	+	+ +	0	0	0	0	0
IV - Bl. (O)	+ +	+ +	+ +	+ +	+ +	0	0	0	0	0
V - Bl. (O)	+ +	hem.*	+ +	+ +	+ +	0	0	0	0	0
Jakob	+ +	+ +	+ +	+ +	+ +	0	0	+	0	0

*Probably hemolysis.

	Marie (A)	Johann (A)	Ludwig (A)	Frau Tr. (A)	Oberin (B)	Wernicke (B)	Deetjen (B)	Ter. (B)	Hirschfeld (O)	Amalie (O)	Kalchschmidt (O)
Chimpanzee:											
I	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
I - Hirschfeld (O)	0	0	0	0	+ +	+ +	+ +	+ +	0	0	0
II	±	±	0	±	+	+	+	+	0	0	0
II - Kalchschmidt (O)	±	0	0	0	±	±	±	±	0	0	0

Persons in Group A	Sera with which the kinds of blood can be differentiated
Marie & Otto	1) Cat 2 - Hirschfeld; 2) Cat 2 - Marie; 3) Horse 1 - Luise, etc.
Marie & Hanna	1) Cat 2 - Hirschfeld; 2) Cat 1 - Ludwig; 3) Cow 6 - Hirschfeld; 4) Cow 5 - Ludwig; 5) Cow 11 - Heizer, etc.
Marie & Anna I	1) Cat 1 - Ludwig; 2) Horse 1 - Luise
Marie & Ludwig	1) Cat 2 - Hirschfeld; 2) Cat 5 - Karl; 3) Cat 1 - Ludwig; 4) Cat 2 - Marie
Marie & Anna II	1) Cat 3 - Caan; 2) Cow 11 - Heizer

Persons in Group A (Continued)	Sera with which the kinds of blood can be differentiated
Marie & Heizer	1) Cat 4 - Hirschfeld; 2) Cat 5 - Karl; 3) Cat 1 - Berstedt; 4) Cow 1 - Elisabeth
Marie & Bochen	1) Pig 1 - Hirschfeld; 2) Cow 8 - Marie
Bochen & Otto	1) Cat 12 - Bochen; 2) Pig 3 - Luise; 3) Cow 8 - Marie
Bochen & Hanna	Cow 8 - Marie
Bochen & Anna I	Pig 3 - Luise
Bochen & Ludwig	Pig 1 - 3 - Hirschfeld
Bochen & Anna II	not investigated together
Bochen & Heizer	1) Rabbit 20 - Elisabeth; 2) Pig 3 - Luise; 3) Cow 1 - Elisabeth
Otto & Hanna	1) Cat 1 - Berstedt; 2) Cat 1 - Ludwig; 3) Rabbit 170 - Wernicke; 4) Horse 1 - Hirschfeld
Otto & Anna I	1) Cat 7 - Deetjen; 2) Cat 6 - Deetjen; 3) Horse 1 - Hirschfeld
Otto & Ludwig	1) Cat 1 - Ludwig; 2) Cat 6 - Deetjen; 3) Cow 1 - Marie
Otto & Heizer	1) Cat 1 - Berstedt; 2) Rabbit 20 - Elisabeth

Persons in Group A	Sera with which the kinds of blood can be differentiated
Otto & Anna II	1) Cat 5 - Karl; 2) Cat 4 - Karl; 3) Goat 3 - Deetjen
Hanna & Anna I	1) Rabbit - Hirschfeld; 2) Cat 9 - Oberin
Hanna & Ludwig	1) Goat 1 - Marie; 2) Pig 1, 2, 3 - Hirschfeld; 3) Cow 5 - Ludwig
Hanna & Anna II	Cat 6 - Karl
Hanna & Heizer	1) Cat 5 - Karl; 2) Goat 3 - Deetjen; 3) Cow 10 - Deetjen
Anna I & Ludwig	Pig 1, 2, 3 - Hirschfeld
Anna I & Anna II	Cat 12 - Deetjen
Anna I & Heizer	1) Cat 7 - Deetjen; 2) Cow 1 - Elisabeth
Ludwig & Anna II	1) Cat 5 - Karl; 2) Cat 6 - Karl
Ludwig & Heizer	1) Cat 1 - Berstedt; 2) Goat 3 - Deetjen; 3) Pig 2, 3 - Hirschfeld; 4) Cow 1 - Elisabeth
Anna II & Heizer	Cat 5 - Karl

Persons in Group B	Sera with which the kinds of blood can be differentiated
Wernicke & Oberin	1) Cat 1 - Oberin; 2) Cow 6 - Hirschfeld; 3) Rabbit 10 & Rabbit 7 - Anna; 4) Dog 21 - Marie; 5) Horse 2 - Wernicke; 6) Dog 23 - Elisabeth
Wernicke & Oberin-Schwester	1) Dog 23 - Bochen; 2) Cow 4 - Elisabeth; 3) Dog 23 - Elisabeth
Wernicke & Deetjen	Cat 30 - Ludwig
Wernicke & Christine	Cat 10 & 13 - Ludwig
Oberin & Oberin-Schwester	1) Cat 1 - Oberin; 2) Dog 21 - Hirschfeld; 3) Horse 1 - Ludwig
Oberin & Deetjen	Pig 2 - Karl
Oberin & Christine	Horse 2 - Wernicke
Oberin-Schwester & Deetjen	not investigated together
Oberin-Schwester & Christine	not adequately tested together
Deetjen & Christine	Cat 8 - Ludwig

Persons in Group AB	Sera with which the kinds of blood can be differentiated
Anna & Luise	1) Rabbit 10 - Marie; 2) Goat 1 - Wernicke

Persons in Group O	Sera with which the kinds of blood can be differentiated
Hirschfeld & Karl	1) Cat 1 - Karl; 2) Cat 9 - Karl; 3) Cat 20 - Bochen; 4) Cat 1 - Oberin; 5) Horse 1 - Bochen; 6) Horse 1 - Anna; 7) Horse 1 - Luise
Hirschfeld & Kalchschmidt	1) Cat 1 - Karl; 2) Cat 1 - Ludwig; 3) Cat 30 - Ludwig; 4) Cat 10 - Ludwig; 5) Cat 1 - Oberin; 6) Horse 1 - Anna; 7) Horse 1 - Luise
Hirschfeld & Berstedt	1) Cat 1 - Karl; 2) Cat 9 - Karl; 3) Cat 2 - Ludwig; 4) Cat 1 - Oberin; 5) Goat 3 - Ludwig; Dog 21 - Wernicke
Hirschfeld & Schwender	1) Cat 1 - Oberin; 2) Young Goat - Hirschfeld
Hirschfeld & Amalie	1) Cat 1 - Ludwig; 2) Cat 2 - Ludwig; 3) Cat 30 - Ludwig; 4) Cat 1 - Oberin; 5) Cat 1 - Karl; 6) Goat 3 - Ludwig

Persons in Group O (Continued)	Sera with which the kinds of blood can be differentiated
Hirschfeld & Elisabeth	1) Cat 1 - Karl; 2) Cat 30 - Ludwig; 3) Horse 1 - Anna; 4) Horse 1 - Luise; 5) Cow 1 - Otto
Karl & Kalchschmidt	1) Cat 1 - Anna; 2) Horse 1 - Bochen; 3) Cat 1 - Ludwig; 4) Cat 20 - Bochen
Karl & Berstedt	1) Cat 1 - Anna; 2) Horse 1 - Bochen; 3) Dog 2 - Wernicke; 4) Cat 1 - Ludwig; 5) Cat 20 - Bochen; 6) Cat 30 - Ludwig
Karl & Schwender	1) Young Goat - Hirschfeld; 2) Cat 2 - Marie
Karl & Amalie	1) Cat 1 - Anna; 2) Cat 20 - Luise; 3) Horse 1 - Luise; 4) Cat 9 - Karl; 5) Cat 1 - Ludwig; 6) Cat 2 - Ludwig; 7) Cat 30 - Ludwig
Karl & Elisabeth	1) Cow 1 - Otto; 2) Cat 30 - Ludwig
Karl & Werner	1) Horse 2 - Hirschfeld; 2) Cat 9 - Hirschfeld; 3) Cat 10 & 13 - Hirschfeld; 4) Cat 9 - Karl
Kalchschmidt & Berstedt	Dog 21 - Wernicke
Kalchschmidt & Schwender	1) Horse 1 - Anna; 2) Cat 2 - Marie
Kalchschmidt & Amalie	1) Cow 10 - Deetjen; 2) Horse 1 - Luise; 3) Pig 2 - Elisabeth; 4) Cat 2 - Ludwig
Kalchschmidt & Elisabeth	1) Cow 1 - Otto; 2) Cat 1 - Ludwig
Kalchschmidt & Werner	1) Horse 2 - Hirschfeld; 2) Cat 9 - Hirschfeld; 3) Cat 10 & 13 - Karl
Berstedt & Schwender	1) Dog 21 - Wernicke; 2) Cat 2 - Marie
Berstedt & Amalie	1) Pig 2 - Elisabeth; 2) Dog 21 - Wernicke; 3) Cat 9 - Karl; 4) Cat 2 - Ludwig
Berstedt & Elisabeth	1) Horse 1 - Anna; 2) Cat 1 - Ludwig
Berstedt & Werner	1) Horse 2 - Hirschfeld; 2) Cat 9 - Hirschfeld; 3) Cat 10 & 13 - Karl; 4) Cat 9 - Karl
Schwender & Amalie	1) Young Goat - Hirschfeld; 2) Cat 2 - Marie

Persons in Group O	Sera with which the kinds of blood can be differentiated
Schwender & Elisabeth	1) Goat 1 - Wernicke; 2) Cow 1 - Otto; 3) Horse 1 - Anna
Schwender & Werner	Examined simultaneously
Amalie & Elisabeth	1) Cow 1 - Otto; 2) Horse 1 - Anna; 3) Cat 1 - Ludwig
Amalie & Werner	1) Cat 9 - Hirschfeld; 2) Cat 10 & 13 - Karl; 3) Horse 2 - Hirschfeld

Persons in Group O (Continued)	Sera with which the kinds of blood can be differentiated
Elisabeth & Werner	not adequately tested together
Caan is more powerfully agglutinated by this serum than the other simultaneously studied blood kinds in the O group	Cow 10 - Hirschfeld
Werner was more strongly agglutinaged by these sera than were the other kinds of blood from the O group	1) Cat 9 - Hirschfeld; 2) Cat 10 & 13 - Karl; 3) Horse 2 - Hirschfeld

When we ask ourselves whether it is possible to establish, with the /542
aid of these animal sera, sufficient individual differences to make it possible to distinguish the individual kinds of blood in the four groups A, B, AB, and O, then the records show that it is indeed possible to separate any sufficiently investigated blood from all the others, and without any difficulty.

It will, however, be noted that differentiation is possible only when /545
the tests are undertaken by means of a sufficiently large number of sera. It cannot be expected that it should be possible to characterize completely unknown kinds of blood with but a few animal sera. We had undertaken such an experiment. About 25 of the sera designated in the records were selected and used to differentiate the kinds of blood of 25 strange patients. This was definitely sufficient to define differences within the individual main categories, but it was not possible to distinguish between all individuals. Very pronounced differences were found in one blood variety both with respect to group A and group non-A, and this blood possessed the properties of a "little A". The records in the table on p. 546 clearly show these differences. The

	Sera								
	Wernicke (B)	Oberin (B)	Bochen (A)	Equine 2-Wernicke (B)	Goat -Christine (B)	Goat 3-Amalie (O)	Dog 13-Deetjen (B)	Cat 6-Carl (O)	Cat 10-Ludwig (A)
				1	2	3	4	5	6
Blood:									
Pat. No. 14 (a)	+ +	+	0	+ +	+	÷	+	+	0
No. 15 (a)	+ +	+ +	0	+ +	+ +	+	+ +	÷	0
No. 6 (a)	+ +	+ +	0	+ +	+	+	+ +	+	0
No. 7 (a)	+ +	+	0	+ +	+ +	+	+ +	+ +	+
No. 16 (small a, b)	+	0	+ +	±	0	±	±	±	±
No. 8 (a, b)	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+
No. 10 (b)	0	0	+ +	0	0	+	0	+	±
No. 12 (O)	0	0	0	0	±	0	0	±	±
No. 13 (O)	0	.	0	+	0	0	0	±	±
No. 22 (O)	0	.	0	0	±	0	0	±	0

small differences in other cases are primarily attributable to the fact that the selected sera were adjusted to quite well-determined kinds of blood. It is therefore hardly possible to use this method in a practical sense for renewed recognition of an individual although, as we have seen, this is possible in principle.

3. CONCERNING GROUP-SPECIFIC STRUCTURES OF THE BLOOD CORPUSCLES OF VARIOUS VERTEBRATES /546

As has already been mentioned, we have conducted investigations concerning the group-specific structures in other animals as well and compared in these terms not only the variations which occur within a species, but also the structures of various animal species with each other. Occupying first place

Sera												
Bovine 11-Heizer (A)	Cat 15-Heizer (A)	Cat 3-Caan (O)	Cat 1-Berstedt (O)	Equine 1-Anna (AB)	Cat 14-Heizer (A)	Goat 3-Deetjen (B)	Equine 2-Carl (O)	Equine 1-Bochen (A)	Oberin (B) - Marie (A)	Cat 2-Ludwig (A)	Goat -Hirschfeld (O)	Goat -Oberin (B)
7	8	9	10	11	12	13	14	15	16	17	18	19
0	0	±	±	0	0	+	+ +	0	0	±	±	+
0	0	±	±	0	0	+	+ +	0	0	±	±	±
0	0	+	+	±	+	+	+ +	±	+	+	.	±
±	+	+ +	+ +	+	+	+ +	+ +	±	±	+	.	.
+	+	±	0	0	±	0	+	0	0	±	+	0
+	+	+	+	0	±	+	+ +	±	0	+	+	+
+	+	+	±	0	+	0	+ +	0	0	+	+	0
+	+	0	0	+	+	0	±	±	0	+	0	0
±	0	0	0	0	±	0	0	0	.	+	0	0
±	0	0	0	+	+	0	0	0	.	+	0	0

was the investigation of whether the structures A and B, which are present in man, can also be found in other animals as well. On that occasion it was necessary to take up anew the question of the species -- specificity of the natural agglutinins, and in addition it was necessary to test to what extent the species-specific agglutinins coincide with the group-specific ones. Some investigations with rabbit blood corpuscles were conducted in a most precise manner by the unfortunately demised cand. med. Leo Saal. A part of the files were lost upon his death, so that we shall cite the results obtained, from our memory. Other animal species were covered by cand. med. Brockmann who will soon report upon his observations in detail.

	Sera									
	Bovine 10-Deetjen (B)	Cat 9-Oberin (B)	Cat 12-Bochen (A)	Bovine 10-Hirschfeld (O)	Goat 1-Wernicke (B)	Cat 30-Ludwig (A)	Cat 2-Marie (A)	Equine 1-Hirschfeld (O)	Bovine 8-Hirschfeld (O)	Cat 2-Hirschfeld (O)
	20	21	22	23	24	25	26	27	28	29
Blood:										
Pat No. 14(a)	+ +	+	0	+ +	+	±	±	0	+ +	+ +
No. 15 (a)	+ +	+	0	+ +	+ +	±	±	±	+ +	+ +
No. 6 (a)	+ +	+	0	+	+ +	±	+	+	+ +	+ +
No. 7 (a)	+ +	+	+	+ +	+ +	+	.	.	.	.
No. 16 (small a, b)	+	0	±	+ +	0	+ +	+ +	0	+	+ +
No. 8 (a, b)	+ +	+	+	+ +	+	+ +	+ +	+	+ +	+ +
No. 10 (b)	0	0	+	+	±	+ +	+ +	0	0	+ +
No. 12 (O)	0	0	+	0	.	.	.	.	.	.
No. 13 (O)	0	0	±	0	0	±	0	0	0	0
No. 22 (O)	0	0	±	0	±	±	0	0	0	0

We employed the absorption method for the investigation of these questions. In order to be able to decide in which mammals the blood behaved in the /547 same way as does human blood of the A or B groups, human sera containing isoagglutinins were charged with animals blood, allowed to remain in contact for one hour, and then tested for the presence of alpha and beta with human kinds of blood. Tests were also conducted in this connection to determine whether human serum will agglutinate animal blood as effectively after absorption of alpha and beta by human blood as it did before. For the chimpanzees we have so far been able to investigate only one kind of blood. This blood possessed human structure A, but not structure B.

This was powerfully agglutinated through the sera of the B group, but not

by the sera of the A group. The absorption of the active sera with a blood from the A group (Johann) gave results which varied with the particular serum being used. The sera of Deetjen, Meyer, Oberin, no longer agglutinated the chimpanzee blood. On the other hand, the Wernicke serum continued to agglut-

te it, although it is true that the agglutination action was weaker. We may therefore conclude that the Wernicke serum contained, in addition to alpha, still another agglutinin which attacks the chimpanzee blood, and which is not effective on any of the investigated kinds of human blood. The blood of /548 the studied chimpanzees, like human blood, thus contains the A structure and in addition also something else that is lacking in human types of blood. Nor was all of the isoagglutinin in the Wernicke serum extracted by the chimpanzee blood, there still remaining sufficient agglutinin for two of the kinds of blood belonging to two A group members, while a third one was no longer agglutinable. Investigation of two of the chimpanzee sera available to us would seem to indicate that these two animals also contained A in their blood, but no true B. Thus the chimpanzee blood can be stated to agree, widely, with human blood of the A group. It therefore became very important, naturally, to investigate the anthropoid ape extensively in this direction and to compare it with the various human races, particularly since the most recent anthropologic investigations, which have been set under way by Melchers and Klatsch, show indications that the human family may be composed of various races, each being close to some other, particular anthropoid.

The blood corpuscles of the four Rhesus monkeys under investigation behaved quite differently. They would become agglutinated by some human sera, and not by others, but it proved impossible to conclude that some relation existed between the agglutinating action and alpha or beta. The Wernicke serum,

when absorbed with the blood of two Rhesus monkeys, lost its agglutinin for the blood of all Rhesus monkeys, but not for that of the chimpanzees. Nor was it possible to establish the presence of any isoagglutinins.

Rabbit blood was tested in a large number of cases; and alpha was never /549 absorbed by the rabbit blood corpuscles, while beta, on the other hand, was absorbed in the majority of cases. Here a remarkable phenomenon was noted: although beta was not absorbed by some of the human sera, the same rabbit blood would capture the agglutinin, that was specific for B blood, from other human sera. When tests would be conducted on a serum of this type with absorbable beta, then this would be captured by the blood of all rabbits; an exception being noted only in the case of two out of more than 30 rabbits. The serum of one of these two animals, after being absorbed with human blood from the O group, no longer affected the blood corpuscles of groups A and B. There was, however, no connection between the absence of a B-active agglutinin in the serum and the fixing capacity of the blood for human beta agglutinin, since we found all possible combinations. Present most frequently is a simultaneous fixation capacity on the part of the blood for beta, and the agglutinating action of the serum for blood B. The rabbit blood corpuscles can be divided by the immuno-isoagglutinins into groups, yet the agglutinins are weak and hence less suitable for analysis.

The sera of other animal species usually acted on all kinds of rabbit blood, and in fact the agglutinins for the blood kinds of other animals vanished after absorption with rabbit blood. Only in rare cases were there /550 group-specific agglutinins present. The extent to which the groups which are detected by various methods overlap or differ was not investigated for the rabbit.

Sera	Chim-panzee	Rhesus I	Rhesus II	Rhesus III	Rhesus IV	Rhesus V
Ludwig (A)	0	0	+	+	+	±
Bochen (A)	0	0	±	0	±	0
Marie (A)	0	+	+ +	+ +	+ +	+ +
Hanna (A)	0	±	+	+	+	+
Johann (A)	0	0	0	0	0	0
Deetjen (B)	+ +	+	+ +	+ +	+ +	+ +
Oberin (B)	+ +	0	0	0	0	0
Wernicke (B)	+ +	+ +	+ +	+ +	+ +	+ +
Meyer (B)	+	0	0	0	0	0
Gorovitz (AB)	0	±	+	±	+	0
Hirschfeld (O)	+	0	0	0	0	0
Carl (O)	+	±	±	±	±	0

Sera	A Johann	Chim-panzee	Chim-panzee	Chim-panzee	Chim-panzee
Wernicke (B) - Johann (A)	0	+	.	.	.
Deetjen (B) - Johann (A)	0	±	.	.	.
Oberin (B) - Johann (A)	0	0	.	.	.
Meyer (B) - Johann	0	0	.	.	.
Wernicke (B) - Chimpanzee	.	0	±	+	0

Sera	Rhesus I	Rhesus II	Rhesus III	Rhesus IV	Chim-panzee
Wernicke (B) - Rhesus III	0	0	0	0	+
Wernicke (B) - Rhesus IV	0	0	0	0	+

We have already reported in this Journal (Vol. 4, 1909) on the groupings of varieties of canine blood which were obtained through the use of immuno-isoagglutinins. Groupings could be proved for the sera of other animal species as well. All of the dogs investigated possessed the property of being able to absorb agglutinin beta, whereas agglutinin alpha remained behind under precisely the same experimental arrangements, the blood of only a single dog being

capable of weakening the agglutination action on the human corpuscles of group A. Investigations conducted with other animal sera likewise showed differences in the absorptive capacities of the individual varieties of blood. Some of the blood corpuscles would extract the total agglutinin, others would leave some residual which would be demonstrable through its agglutination action on other varieties of blood. There was no connection between this connection and the specific components which we detected through the use of isoagglutinins.

The bovine blood corpuscles were not agglutinated by human sera, yet they would absorb the human betas but not the alphas. An absorption capacity with respect to beta was present even when the bovine serum reacted powerfully with human blood of the B group. This agglutinin of the bovine serum (e.g., bovine 4-Hirschfeld) was not fixed by the corresponding bovine blood. One bovine blood constituted an exception to this extent, that it was agglutinated by some individual human sera. The agglutinin to which reference is being made here has nothing to do with alpha and beta. The deficiency in the agglutinability of bovine blood corpuscles makes impossible a differentiation with the aid of normal agglutinins. According to the observations of Todd and White, however, (Proceedings of the Royal Society, June 1910; Journal of Hygiene, September 1910) who conducted their studies with immuno-isohemolysins, very extensive individual differences exist.

Only a few experiments were conducted using cat and equine blood cor- /551
puscles. At most, beta absorption by these blood corpuscles was only partial, and alpha was not absorbed at all.

It can therefore be said that the blood corpuscles of numerous animal species can absorb human agglutinin beta. This fact led us to investigate the extent to which agglutinins are in any way species-specific. The sera of a

number of vertebrates were absorbed with the blood of various animal species, and then tested for their actions on the blood of other animals and humans.

Sera	A	B		Non-A, Non-B						
	Marie	Oberin	Deetjen	Kalchschm.	Hirschfeld	Scheck	M.	Carl	Amalie	Elisabeth
Cow	+ +	+ +	+	±	±	+	±	±	±	±
Cow-rabbit 221	+ +	+	±	0	0	0	0	0	±	±
Cow-dog (17 & 13)	+ +	+	±	0	0	0	0	0	0	0
Cow-pig	±	+	±	0	0	± 0	0	0	±	0
Cow-rabbit 412	+ +	+	+	±	±	±	0	±	±	±
Pig	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Pig-rabbit 221	+ +	+ +	+ +	+	+	+ +	+	+ +	+	+ +
Pig-rabbit 412	+ +	+ +	+	+	+	+ +	+	+	+	+
Pig-dog (17 & 13)	+	+	+	+	+	+	+	+	+	±
Pig-rabbit 381	+	+	+	+	+	+	+ +	+	+	+
Goat	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Goat-pig	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Goat-cow	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+	+ +
Setter 27	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Setter-pig	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Setter-cow	+	+ +	+	+	+	+	+	+	+	+ +
Setter-rabbit 412	+ +	+	+	+	+	+	+	+	+	+
Setter-rabbit 121	+ +	+ +	+	+ +	+	+ +	+	+	+ +	+

The experiment shows that the animal-species bloods employed did not, for the most part, absorb the agglutinin which acts on human blood. Yet the bovine serum which was investigated here found its agglutination action on human blood decreased to a not inconsiderable extent by contact with rabbit, pig and canine blood. This attenuation cannot be attributed to the presence of A or B in these animal blood corpuscles. Studies with animal blood sera, which had been conducted by Brockmann, led to similar results. In agreement with the ob- /552
servations of Malkoff, it proved possible to detect specific agglutinins for

the blood of various mammals (sera of the horse, dog, cow, goat, blood corpuscles of rabbits, cattle, dogs, pigs, goats). The agglutinins of the human sera agglutinins of the human sera studied. The absorption action covered numerous kinds of blood; thus, for example, rabbit blood extracted the total canine-blood agglutinin from the serum. Some kinds of fowl blood (chicken, pigeon, duck, goose), on the other hand, did not extract the agglutinins which react with the blood of the various mammals, yet groups of bird species' bloods could be found which would react with a given agglutinin in human sera. Therefore, there do exist agglutinins which are highly species-specific and at the same time there are those which will react with the blood corpuscles of numerous species.

It was further investigated whether species-agglutinins of human sera for the blood of various vertebrates are identical with agglutinins alpha and beta. Experimentation showed that this is not the case, that after the removal of alpha and beta through a suitable kind of blood there were no changes created in the agglutinins of the investigated types of animal blood. The agglutinins alpha and beta cannot be identified with any of the agglutinins which act on these foreign varieties of blood.

4. ON GROUP-SPECIFIC IMMUNO-AGGLUTININS

As we have s n, pretreatment of animals with the blood of the same animal species gives rise to group-specific agglutinins. We can state further that group-specific agglutinins for human blood are almost regularly to be found in most of the animal species. It therefore seemed proper to investigate whether it is also possible to cause group-specific agglutinins to develop anew following pretreatment with the blood of foreign animal species. We investigated this question in animals which had not contained any group-specific

agglutinins in their serum before the pretreatment. This occurs extremely rarely in rabbits, and not too frequently in dogs. We have therefore pretreated to date only one rabbit and one dog with blood from the B group (Deetjen blood). Both animals behaved in the same way: following pretreatment, /553 their serum very markedly agglutinated all sorts of human blood; but bloods from group B were agglutinated at even greater dilutions. Following repeated absorption with a blood from the O group there remained behind an agglutinin which worked only on individual kinds of blood which belonged to group B.

Serum from Setter 27	1/2	1/4	1/8	1/16	1/32
On blood from group A					
Before immunization	±	0	.	.	.
Following the first injection	+ +	+	±	+	.
Following the second injection	+	±	±	±	±
On blood from group B					
Before immunization	±	0	.	.	.
Following the first injection	+ +	+ +	+	±	±
Following the second injection	+ +	+ +	+	+	±
On blood from group O					
Before immunization	±	0	.	.	.
Following the first injection	+	+	±	±	.
Following the second injection	+	±	±	±	±

Another dog received an i.p. injection, by Dr. Brockmann, of the blood /554 of yet another dog. Isoagglutinins occurred for the blood of this dog and strikingly the serum also developed the ability, following absorption with human blood from the non-B group, to agglutinate blood varieties from the B group, while this action was definitely not present before injection of the

canine blood. This agglutinin was not absorbed from the serum through the dog's blood used in the pretreatment; it is, therefore, in no way identical with the isoagglutinin, although it did occur in connection with the injection of the canine blood.

The serum after absorption with Hirschfeld blood (0)	A			B[1]		0	
	Johann	Ludwig	Ida	Oberin	Deetjen	Hirschf.	Brock-mann
Setter 27 before injection	0	0	0	0	0	0	0
Setter after first injection	±	±	±	+ ±	+ ±	0	0
Setter after the second injection	0	±	0	+ +	+ +	0	0

Serum after absorption with Hirschfeld (0) blood	Hirschfeld	Ludwig (A)	Deetjen (B)[1]
"Silver" rabbit before immunization	0	0	0
After immunization with Deetjen blood (B)	±	±	+ +

5. CONCERNING GROUP-SPECIFIC IMMUNO-AGGLUTININS AGAINST TISSUES

The fact that group-specific antibodies can also occur in animals that have received blood from a foreign animal species, gives us a way of analyzing the group-specific structures of human tissues.

Our experiments in this subject have not yet been completed. Yet we should like to report two dominant constituents in connection with our earlier

[1] Agglutination occurred very rapidly.

studies concerning individual isoantibodies which we were able to obtain following injection of dog kidneys into other dogs. At that time we were able to distinguish, through the isoagglutinins in the blood of our dogs, two dominant constituents. One of these was found in the father dog and offspring 4, the other in the mother dog and offspring 3 and 4. The blood that contained neither did not give rise to the formation of any isoantibodies. We therefore sacrificed one dog who possesed such blood (offspring 1), removed the kidney, made a fine suspension out of this, and injected this into a number of dogs whose blood belonged to various groups, into the abdominal cavities of others (father, mother, offspring 2, 3 and 4). The serum of the pretreated dogs was then studied on January 28 and February 5. All of the sera agglutinated the mother's blood, except the serum of the mother herself. The other kinds of blood were not agglutinated. Thus, injection of renal tissue of an animal whose blood contained neither the maternal nor the paternal blood antigens, was in all /555 cases followed by the formation of an agglutinin for the mother's blood. The specific substances in the maternal blood which reacted with these agglutinins cannot be the same as those which combine with the agglutinins produced by injection of blood. The blood of offspring 3, which behaved in exactly the same way as the mother's was not agglutinated in this case, in fact, the serum of offspring 3 acted, after pretreatment with the kidney suspension, most strongly upon the maternal blood. Remarkably, the renal suspension proved incapable of extracting the agglutinin from the serum. We also investigated the sera with the complement-diversion method. The renal tissue, along with the serum of the mother dog, had a total and durable inhibiting action, the same being true for the serum of offspring 3, while inhibiting was quite low with the paternal serum, and absent completely with the serum of offspring 4. The same grouping was

manifest here as when the same component had acted as an antigen in the kidney, and which had been discernible in our earlier experiments with the blood of the father and of offspring 4. The test for agglutinins and for complement-diverting substances thus lead to quite different results.

6. THEORETICAL CONSIDERATIONS

As we have seen, the blood corpuscles of the various animal species possess numerous properties, which are not to be found in all of the individuals of a given species. These properties are suitable for characterizing the individual. At least in man we conducted repeated investigations on one and the same person, and always found the same properties to be present. Even such refinements as were demonstrable through the absorption methods in blood varieties in the same group, were clearly manifest in the numerous repetitions. To be sure, our experience is extensive only with respect to properties A and B, and here the fact that Mendel's law was found to hold with respect to their inheritance speaks for the constancy of the particular organization of the structures.

We obtained a whole multitude of different agglutinins through absorp- /556
tion of animal sera, and were able to conduct a whole series of further differentiations with their aid. In the majority of cases the groupings arose, as has already been mentioned, according to properties A and B. We must therefore ask ourselves whether the animal-serum agglutinins being considered here are identical to the human agglutinins alpha and beta.

The numerous records which we have presented do furnish information on the subject up to a certain point. After absorption with a specific human blood the agglutination action of the animal sera would in general be either abolished or maintained for the blood varieties of a group A or B, depending on whether the blood used for absorption did or did not belong to the correspond-

ing group A or B. We would therefore conclude that an identity did exist, if there were no exceptions. It proved however to be the case, almost invariably, that out of a given number of bloods from the A or B group, one or another would break precedent and not be agglutinated. It would also happen that individual blood varieties from the non-A, non-B group would behave the same as the majority of the bloods that contained A or B; but this is less convincing since one can take the existence of yet another agglutinin to be the explanation for this. It therefore appears that there does not exist complete identity between the animal sera which are directed against A or B blood and the alphas and betas of the human sera. The experiments show further that there exists a large number of agglutination actions; which, despite their differences, all influence the bloods of the same A or B group. It does not however follow from these observations that in principle the animal agglutinins are to be contrasted with human ones, since we were able to demonstrate the existence of similar, albeit milder, phenomena, when the various human sera were being permitted to act.

We therefore undertook a further comparison of animal and human agglutinins, with the aid of absorption experiments employing animal blood. As has already been reported, human agglutinin beta is readily absorbed by the blood of numerous animal species. We therefore experimented also, in the same /557 way, with animal sera which had exerted a particularly powerful effect on the blood varieties of the B group. The first such experiment was performed with rabbit serum. The agglutinin that acts upon human blood in the B group remained entirely in the serum after having been placed in contact with rabbit and bovine blood. Additional experiments were undertaken with three canine sera which were particularly powerful agglutinants of B blood.

Sera of	Before Absorption With Animal Blood	Absorption With Bovine Blood	Absorbed With Blood From Dog 27	Absorbed With Blood From Dog Fochs
Dog 17 (immunized with blood from dog 27)-Hirschfeld blood	+	+ - ±	+ - ±	+ - ±
Dog 27 (immunized with Deetjen blood, B)-Hirschfeld blood	+	+ - ±	+ - ±	+ - ±
Dog Fochs with agglutinin that acts powerfully on b-Hirschfeld blood	+	+ - ±	+ - ±	+ - ±
Ludwig (A)	+	0	0	0

Tested on group B (Oberin) blood corpuscles

It was found that none of these agglutinins from the blood of two dogs and one cow were absorbed. From this experiment it would seem that there is a pronounced contrast between the agglutinins of human and animal serum. But this is somewhat weakened by the fact that the beta from some human sera is also not absorbed by animal blood. It was therefore necessary to consider the possibility that the variations between sera are not due to the agglutinins themselves, but to other substances that accompany them. However, it was not possible to provide experimental support for this assumption. Addition of rabbit serum, which has but a weak effect on B blood, did not impede absorption of human beta by rabbit blood; thus it proved impossible to demonstrate the presence in the rabbit serum of any substance which would impede the absorption of beta by rabbit blood. Nor was it possible, by adding beta-containing human serum (Ludwig serum), to encourage the absorption by canine and bovine blood, of the agglutinins from dog serum that acts on B blood.

	Absorption With Bovine Blood	Before Absorption
Serum from dog 17-Hirschfeld blood + Ludwig serum	+	+ +
Serum from dog 27-Hirschfeld blood + Ludwig serum	+	+ +

	Absorption With Blood From Dog 27	Before Absorption
Serum from dog 17-Hirschfeld blood + Ludwig serum	+	+ +
Serum from dog 27-Hirschfeld blood + Ludwig serum	+	+ +

Sera from dog 27-Hirschfeld (0), and dog 17-Hirschfeld, were mixed in /558 equal parts (0.25 each) with Ludwig (A) serum, with 0.5 blood from dog 17, dog 27, and bovine, and allowed to remain in contact for one hour, and then tested for effectiveness on blood B (Deetjen).

It appears therefore that the human sera contain no substances which promote absorption of animal beta. One must also assume that the analogous agglutinins in the various sera are not identical, even if there is no difference in principle.

In analyzing the various agglutinins it should also be considered that not all of the effects which one may observe need to be based on preformed substances. Our investigations show very clearly that changes in the serum occur after absorption, and that it is impossible to attribute these to a simple ex-

traction of certain given agglutinins. When a given serum is absorbed with various kinds of blood and the residual agglutination effects for the numerous kinds of blood are compared with each other, then it appears that the results which have been obtained are contradictory, if one assumes a simple removal of certain given agglutinins. For example: Hirschfeld blood will remove from equine serum all of the agglutinins for Karl blood, but leaves behind an agglutinin for Otto blood. This means that Otto blood reacts with an agglutinin which has no relation to the Karl and the Hirschfeld bloods. Yet in a second experiment, Karl blood removed this agglutinin from the serum. In certain individual cases we observed directly that after certain agglutinins had been absorbed from the serum, new agglutination actions would develop which had /559 not existed before.

Sera of	Faust	Frau Schade	Gertrud Schade	Herr Kröger	Friedrich Kröger	Georg Kröger
Wernicke (B)	0	0	0	0	0	0
Wernicke-blood Knoll (little A)	±	±	±	±	±	±

It must therefore be assumed that new systems can form during absorption. Whether the agglutinins had been formed earlier was something that for the most part we could not determine for the individual instances. But that this is so is highly probable, for some of them, since some of the animal sera preferentially agglutinated blood varieties from groups A or B, even before absorption with human blood.

Thus the numerous agglutinins in the various sera make it possible to detect many specific properties of the blood corpuscles. Some of these pref-

erentially occur in sets, and in fact simultaneously with properties A and B, in such a way that the difference between A and little A is manifest. Other properties, on the other hand, proved to be independent of these groupings. There exist, then, genetic units which are transmitted to the progeny in accordance with the Mendelian laws. We are not yet in a position to report definite results concerning inheritance of the properties which can be demonstrated through animal sera. In the case of one property which could be characterized without absorption with the aid of a certain monkey serum, independent heredity could be established.

	Mother	Father	F	F	M	F
Serum A (β)	0	+	+	0	+	+
Serum B (α)	0	±	±	0	±	±
Serum from a monkey (γ)	0	+	0	+	0	0

However, a large fraction of the specific properties that can be characterized with the aid of animal sera appear to be inherited not separately, but collectively, together with the properties which are defined by human agglu- /560 tinins. Support for this view is given by their joint occurrence. The few investigations which we have conducted for families also indicate that many specific properties occur or fail to occur in the children. We thus see that the properties which are characterized by the agglutination action of the various sera belong in part to different genetic units, but it is also partly true that various properties are represented by one and the same genetic unit. The reason for the fact that certain entities together form a genetic unit and others do not, is something that cannot be decided at present. All that the observations can tell us now is that there is a factor present which combines a

number of properties.

We have considered the possibility that the different bloods of groups A and B possess special agglutination-favoring principles, each of which make a certain number of different substances capable of reacting with various agglutinins. Thus, a different activator will correspond to each genetic unit. Instead of assuming specific activators it would appear that it would be equally proper to introduce principles which, for blood varieties from groups non-A and non-B, inhibit agglutination of a given number of substances. We were unable to provide any experimental basis for these viewpoints.

We can also do what we have done before, namely, we can picture the relations in the guise of complex structures. The genetic unit corresponds to the main structure of the entire substance. The differences in individual parts cause the differences in the behaviors of the individual agglutinins. Non-A and non-B must, therefore, also be separate substances, but it is impossible to state whether the differences in relation to A and B are based upon variations in the basic structure or upon a large number of smaller differences.

SUMMARY

1. The normal isoagglutinins characterize structures A and B in the human /561 blood corpuscles; these being inherited independently of each other in accordance with the Mendelian laws. It was found that these are not always uniform. Differences may be more clearly shown partly through use of different human sera, and partly through absorption of a serum with a given kind of blood.
2. Animal sera also possess group-specific agglutinins for human blood, but these first come to light after the serum has had all the agglutinins that act upon all human blood varieties removed by absorption with a given human

blood. The results presented in the numerous records shown here indicate that numerous specific properties can be recognized with the aid of this method.

3. The groupings of the various kinds of blood which arise in this connection are for the most part the same as those that occur under the action of human sera. The sera, then, behave as though they contained alpha and beta. In other cases it is also possible to demonstrate an elective action on those blood kinds which contain neither A nor B. In addition, differences are found which point to variations in the individual structures which are characterized by the isoagglutinins. It may happen that a given blood with an A or B structure will behave differently towards an animal agglutinin than will another variety of blood from the same group; and will show agreement with individual kinds of blood which contain non-A or non-B. The behavior of sera from individual animal species is discussed in detail, and the possibility of individual blood diagnoses is indicated.

4. The investigation with respect to group-specific structures was extended to the blood of other vertebrates. The specific structures were compared with human ones. It was found, inter alia, that structure A is found in chimpanzees only, while structure B is encountered in a series of vertebrates. In addition, still other structures may be shown to be present in rabbits and dogs, with the aid of isoagglutinins.

5. This capacity on the part of animal blood varieties, which correspond to the blood varieties in group B, to absorb beta, can be demonstrated for /562 most but not all beta-containing sera. It therefore follows that the analogous agglutinins do not need to be identical. This also explains the peculiar phenomenon that most animals do contain blood corpuscles which

will absorb human beta and yet have an agglutinin in the serum which will act specifically on human bloods from the group.

6. The question of the species-specificity of the natural agglutinins is discussed again. It was found, inter alia, that especially in human serum there are agglutinins present which are absorbed in the same way by the blood of quite different animals. These are not identical with the group-specific agglutinins alpha and beta. The agglutinins of the studied animal sera in general show a species specificity. However, in individual cases the agglutinins for human blood are also absorbed by animal blood.
7. In such animals whose serum contains no agglutinin for human blood of the B group, it is possible, through pretreatment with human blood from the B group, to induce the corresponding group-specific agglutinin. One dog even supplied such agglutinin after injection of canine blood.
8. The experiments were extended to tissues and some results reported thereon.
9. Also discussed, and related to a uniform principle, is the striking phenomenon that many structures which are characterized by different agglutinins, usually occur jointly. The relation of these structural elements to genetic units are explained.

RESULTS OF A BIOSTATISTICAL, SUMMARIZING STUDY OF HEREDITARY HUMAN BLOOD STRUCTURES

Felix Bernstein

Translation of "Ergebnisse einer biostatistischen zusammenfassenden Betrachtung über die erblichen Blutstrukturen des Menschen". Klinische Wochenschrift 3(33): 1495-1497, 1924.

U. S. ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX KENTUCKY 40121

RESULTS OF A BIOSTATISTICAL, SUMMARIZING STUDY OF HEREDITARY HUMAN BLOOD STRUCTURES

Felix Bernstein

Mathematical-biological analyses of experiences with hereditary blood structures demonstrates the following: */1495

1. The gene hypothesis of two independently mendelizing pairs of genes is untenable and must be replaced by the hypothesis of three multiple allelomorphs, A, B and R. The four observed blood classes have the following hereditary composition:

Class:	O	A		B		AB
Formula:	RR	RA	AA	RB	BB	AB

RR, AA, BB are the original classes resulting from mutation, and RA, RB and AB are the mixed classes resulting from cross-breeding.

2. We are dealing with three races, of whom the American Indians and the Philippinos still represent the almost pure RR characteristic. Mathematical analysis makes it possible to calculate the frequency of the three races at every geographical location (see table!). It is evident that the R race is still the predominant one in every country, and that the A race and the B race, which was formed in Europe and East Asia or India, occurs mixed with a great remaining mass of unmutated RR individuals. Presumably, the formation of the pure B race occurred in the Malayan Archipelagos, corresponding to the hypothesis that the Japanese, who constitute the majority (see No. 4) resulted from a mixture of Malayans and Mongolians; this constituent simultaneously

---

*/Numbers in the margin indicate pagination of the original foreign text.

diffused into India, and from there eastward, reaching England, where it is still found with a frequency of 5.2%. On the other hand, the A race, predominant in Europe, was mixed with another large portion of an unmutated race, and penetrated into the East to India, where we still find 14.9%. Thus, there is a European-Indian connection, as well as an Indo-European connection (see Hirszfeld: Klin. Wochenschr. No. 26, 1924.). At the same time, the Manchus show a stronger influence of the A characteristic than other Mongolian peoples, so that the A race points to a connection with the North.

3. Division shows two independent percentages, p and q. Since 4 blood classes with 3 independent percentages have been observed, there must be a relation between the latter. According to the hypothesis of independent pairs of genes, the relation is the following: $(A + B)\cdot(B + AB) = AB$, according to our hypothesis:

$$\underbrace{I - \sqrt{B + 0}}_{p} + \underbrace{I - \sqrt{A + 0}}_{q} + \underbrace{\sqrt{0}}_{r} = I$$

The latter relation is most accurately confirmed by the tables, whereas the former shows strong deviations; e.g., according to Kirihara, the following are the results for Japanese in Korea (502 persons):

$$A + AB = 50\% = 0.500,\ B + AB = 28.4\% = 0.284$$

$$(A + AB)\cdot(B + AB) = 0.142,$$

whereas $AB = 7.8\% = 0.078$.

Thus, a convincing proof for the correctness of the hypothesis of multiple allelmorphs is established.

4. The race-index of Hirszfeld, A/B, has only momentary significance. Only the values p, q and r, with the above formulas, have final significance. In the East-Asian investigations, with the exception of those of Liu and Wang,

the nomenclature A and B is doubted by us, and is considered likely to be exchanged with the European nomenclature because there was no continuity established with European test sera*.

5. In all the family studies there are only very few contradictions of our hypothesis, and even those are in declining numbers; e.g., Kirihara has found two contradicting cases in a study of 139 families comprised of 611 members, whereas in a recent work by Jervell, no contradiction is found. The medical-forensic application of our hypothesis thus seems undoubtedly justified and could now be made more accurate on the basis of the correct gene schema.

6. The hereditary phenomena and the statistical results, according to the here-proven three-race theory, have the significant consequence that the agglutinogens must be considered to hold a primary position over the agglutinins. One must necessarily assume that the hereditary factors, whose mutation resulted in the three races, have an immediate effect on the agglutinogen of the cells, whereas one may or may not assume that the serum in the three races undergoes no alteration with respect to the agglutinating group, while other alterations still remain possible and even probable. The only assumption which must be made is that some kind of protective action prevents agglutination of an agglutinogen, e.g., the A agglutinogen in the blood cell by the α-agglutinin in the serum: either one assumes a "deflective" protection, as is done by Ehrlich and Morgenroth, who assume an equilibrium between agglutinogen A (which

---

*This assumption is uttered with the necessary reservation. The first work by Hara and Kobayashi was not available to us. In the works with which we have become familiar (Fukamachi), the only decisive criterion, i.e., the comparison with European test sera, has never been mentioned. If, however, the nomenclature should be correct, one would have to look for a connection between the occurrence of the A race in Japan, and its yet unexplained occurrence in Australia.

is also produced by tissue cells) and the simultaneously produced α of the serum, or one postulates the production of a special protective colloid, preventing anto-agglutination. The schema of Landsteiner shows a reciprocity of of agglutinogen and agglutinin, which is merely an apparent reciprocity, which is primarily due to the recessivity of the R factor.

7. The observations of Guthrie and Huck and Coca and Klein, which are /1496 presumably attributed with the discovery of new hereditary factors, can probably be interpreted as proof of the division of class A into RA and AA. One abnormal family, whose family tree is described by Guthrie and Huck, according to our interpretation has a pathologically mutated A gene.

A more detailed study, including a three-race chart, will appear in the Zeitschrift für Induktive Abstammungs und Vererbungslehre.

Addition during correction: 1. In a study by Hirszfeld, which appeared in this journal (No. 26, 1924), and with which we became familiar during the corrections, Hirszfeld has treated the same question which we were studying, i.e., whether the hereditary structure is attributed to the blood cells or to the serum. The following are our reasons for considering the nature of the serum as not being primarily dependent on the hereditary structure, but rather as secondarily altered through the existence of a deflective protection:

The available class divisions according to hereditary blood structures, show the 0 class to certainly be homozygotic. This also corresponds to the heredity formula RR in its hereditary agglutinogen quality. The agglutinogen quality α, β can only be explained with a heterozygotic heredity formula if the agglutinins are produced directly and genotypically. However, a heterozygotic heredity formula is impossible, as according to the observations, this class is not divided and therefore is certainly homozygotic. On the other hand,

being divided and thus being heterozygotic, the AB class is transmitted, whereas the nature of the agglutinin would point to homozygotism if we would assume the hereditary structure to be attributed to the serum. The occurrence of an α- or β-agglutinin in BB or AA children from a marriage of two AB parents, becomes understandable if the agglutinins are directly due to hereditary factors which were generated by the parents, since these factors lack the agglutinins. It is therefore obvious that the specificity of the serum is only due to a pheno-typical lacking of agglutinins and the genotypical characteristics are merely to be constructed from the occurrence of the agglutinogens.

2. Through the Hirszfeld's study we became familiar with the statistics of Tebbutt and Connel, concerning Australians. Appropriate calculation yields the following results:

TEBBUTT AND CONNEL. 1176 AUSTRALIANS

O	B	A	AB	p	q	r
52.6	8.5	36.9	2.0	21.8	5.4	72.5

with the satisfactorily fulfilled relation: $p + q + r = 99.7$

It is well known that in the very complicated population pattern of Australia, we can find Mongolian, Negroid and probably also Indo-European traits, in part superimposed on each other and in part isolated. Therefore, a better evaluation of the results must depend on the knowledge of the origin of the Aborigines studied.

3. The very important discovery made by Hirszfeld, concerning the connection of group specificity with immunity to diptheria, according to the described concept of heredity cannot be due to an hereditary serum structure, but rather must directly be due to the hereditary characteristics of the cells and the secondary influence of the cells on the nature of the serum. The

THREE RACE TABLE (SELECTION)

Investigator	Individuals Studies: Number	Nationality	Class (%) O	B	A	AB	A race p	B race q	R race r	p+q+r
L. and H. Hirszfeld	500	Englishmen	46.4	7.2	43.4	3.0	26.8	5.2	68.1	100.1
"	500	Frenchmen	43.2	11.2	42.6	3.0	26.2	7.4	65.7	99.3
"	500	Italians	47.2	11.0	38.0	3.8	23.7	7.7	68.7	100.1
"	500	Germans	40.0	12.0	43.0	5.0	27.9	8.9	63.2	100.0
"	500	Austrians	42.0	10.0	40.0	8.0	27.9	9.5	64.8	102.2
"	500	Serbs	38.0	15.6	41.8	4.6	26.8	10.7	61.6	99.1
"	500	Greeks	38.2	16.2	41.6	4.0	26.2	10.7	61.8	98.7
"	500	Bulgarians	39.0	14.2	40.6	6.2	27.1	10.8	62.4	100.3
"	500	Arabs	43.6	19.0	32.4	5.0	20.9	12.9	66.0	99.8
"	500	Turks	36.8	18.6	38.0	6.6	25.6	13.6	60.7	99.9
"	500	Russians	40.7	21.8	31.2	6.3	21.0	15.2	63.8	100.0
"	500	Jews	38.8	23.2	33.0	5.0	21.3	15.3	62.3	98.9
"	500	Madagascans	45.5	23.7	26.2	4.5	16.8	15.4	67.5	99.7
"	500	Senegal Negroes	43.2	29.2	22.6	5.0	14.9	18.9	65.7	99.5
"	500	Anamites	42.0	28.4	22.4	7.2	16.1	19.8	64.8	100.7
"	500	Indians (India)	31.3	41.2	19.0	8.5	14.9	29.1	55.9	100.9
F. Verzár and O. Weszeczky	385	Gypsies	34.2	38.9	21.1	5.8	14.5	25.6	58.4	98.5
Jervell		Norwegians	35.6	10.3	49.8	4.3	32.3	7.6	59.7	99.6
Fukamachi*	199	Manchus (Mukden)	26.63	26.63	38.19	8.54	27.0	19.5	51.5	98.0
Hara and Kobayashi*	353	Japanese (Nagano)	24.0	40.5	16.0	20.0	19.7	36.8	49.0	105.5
Liu and Wang	1000	Chinese	30.0	34.0	25.0	10.0	20.0	25.8	54.7	100.5
Coca and Deibert	862	Indians (American)	77.7	2.1	20.2	-	10.7	1.1	88.0	99.8
Cabrela and Wade	231	Philippinos	64.7	19.6	14.7	1.0	8.2	10.9	80.4	99.5
Fukamachi*	363	Koreans	28.09	32.78	26.44	12.67	22.0	26.2	53.0	101.2
Kirihara*	354	Koreans (Heikoku)	30.5	27.4	34.5	7.6	23.9	19.4	55.2	98.5
Kirihara*	311	Koreans (Keiki)	27.3	32.8	32.8	7.1	22.5	22.5	52.2	97.2

*In the work of the authors marked with *, the designations A and B have been exchanged.

rule of heredity which explains the connection with group specificity is that in a mating of Schick-positive and Schick-negative parents, those children with the group of the positive parent will be positive, and those with the group of the negative parent will be Schick-negative. Due to the paucity of investigative material, one may say that the inheritance of the positive trait will occur "most of the time". Accordingly, the following assumption is probably: some of the A, B and R genes probably cause a negative reaction and others a positive reaction to the Schick test, the negative reaction being predominant. Hirszfeld's observations can be explained if the genes can be divided into 2 groups, e.g., A and R on the one hand, and B on the other, while the frequency of the positive and negative Schick reactions is arranged as an inverse frequency ratio. It is obvious that in an extreme case, viz., if all the genes of one kind would be Schick-negative, the others positive, one would have to conclude with exactly the same rules as did Hirszfeld. Of course, this effect of the heredity factors would not make itself felt in the blood cells, but in those cells from which the serum is generated, an assumption whose necessity we arrived at above in a somewhat different way. It is sufficient to assume that a normally present diptheria-immunity portion of the serum is somehow absorbed by the positive genes, in statu nascendi, and is thus rendered ineffective, while the Schick-positive genes leave the diptheria immunity agent in /1497 the serum. Thus, this agent is probably not identical to the agglutinins which Hirszfeld has already shown with the occurrence of Schick-negative AB individuals, who do not possess the class characteristic agglutinins, α and β. (From the Institute for Mathematical Statistics, University of Göttingen).

A SUMMARY OF CONSIDERATIONS CONCERNING THE GENETIC BLOOD STRUCTURES IN MAN

Felix Bernstein

Translation of "Zusammenfassende Betrachtungen über die erblichen Blutstrukturen des Menschen". Zeitschrift für Induktive Abstammungs- und Vererbungslehre 37: 237-270, 1925.

U. S. ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX KENTUCKY 40121

A SUMMARY OF CONSIDERATIONS CONCERNING THE GENETIC BLOOD STRUCTURES IN MAN

Felix Bernstein

INTRODUCTION

Landsteiner [1] had discovered an agglutination phenomenon in human */237 blood which, following complementary studies by v. Descastello and Sturli [2] and Jansky [3], led to a subdivision of mankind into four different classes. Reference is being made to agglutination of the red blood corpuscles of an individual by means of the blood serum of another individual. The observable phenomena may most simply be presented according to the following outline:

FIGURE I

Cell	Serum			
	0	B	A	AB
0	-	-	-	-
B	+	-	+	-
A	+	+	-	-
AB	+	+	+	-

Mankind is thereby subdivided into classes 0, B, A, AB. The symbol "+" indicates that agglutination occurs between the serum of the human class listed in the column headings, and the erythrocytes in the first column, while the symbol "-" indicates absence of such a reaction. To classify mankind in accordance with the above observations requires only test sera A and B, with which the

---

*/Numbers in the margin indicate pagination of the original foreign text.

erythrocytes of any arbitrary individual are placed in contact, since the plus-minus combination makes possible a clear-cut differentiation between the four groups. The term, "Landsteiner's Rule", will be used specifically to des- /238 ignate the characteristic which is found here, namely, the fact that blood corpuscles in Class A are agglutinated by the serum of Class B, etc. Concerning the technique of such tests see particularly Ottenberg's bibliography in J. Exp. Med. 13: 425, 1911, and Jervell and Kirihara.

Von Dungern and Hirszfeld [4] came first to the conclusion, based on hereditary observations of 72 families in Heidelberg, that the specific blood structures are inherited in accordance with the Mendelian genetic laws. The genetic hypothesis corresponding to this was formulated by Ottenberg, who presents the following outline.

FIGURE II

Class	Individual			
0	aa bb			
B	bb BB	bb Bb		
A	AA bb	Aa bb		
AB	AA BB	Aa BB	AA Bb	Aa Bb

In this case, A and B designate dominant genetic factors in the Mendelian sense. The recessive factors are considered by Ottenberg to be the creators of the specific substances (agglutinins) in the serum, whose presence has been hypothesized by Landsteiner.

L. and H. Hirszfeld [6] have presented the percentages of the group-specific structures in the blood of Englishmen, Frenchmen, Italians, Germans, Austrians, Serbs, Greeks, Bulgarians, Arabs, Turks, Russians, Jews, Madagascarians, Senegalese Negroes, Anamites, and Indians, and found hereditary differences. A large number of studies have appeared in this connection (see bibliography). F. Verzár [7] has shown that the percentages for Hungarian gypsies agree with those of Indians, and that the same is true for the German colonists in Hungary and the Germans in Germany, so that the constancy of specific structures appears to be assured over centuries.

In order to be able to obtain a comparative view of the various human /239 races, Hirszfeld formed an index (A/B), and in this way determined a particular sequence. The analysis performed below shows:

1. The A/B index of Hirszfeld's is not valid and its use should be stopped.

2. The genetic hypothesis of two independent Mendelian pairs of genes is not valid, but must be replaced by the concept of three multiple allelomorphs (for explanation see below).

3. We are dealing with a mixture of three races, which we shall designate as the A race, the B race, and the R race (the R race being a summary designation of prototype races), so formed that it is possible to calculate the numerical percentage of each of these three races at every terrestrial observation point. It is necessary to introduce the concept that the A race and the B race are mutations, and in fact apparently recent mutations of the R race, this latter even today is represented in a relatively pure form by the American Indians and the Philippinos.

4. The inheritance phenomena and the statistical results, according to

the three-race theory which is proven here, have the significant consequence that a primary position must be ascribed to the agglutinogens in relation to the agglutinins. It is necessary to assume that the genetic factors whose mutations produced the three races induce changes in the cellular agglutinogens exclusively, while one may be permitted to assume that the serum experiences no change with respect to the agglutinating groups of the three races while other species-specific changes are quite possible, and, indeed, appear probable. The only assumption which must be made here, as well, is that there is some prophylactic action which hinders the auto-agglutination of an agglutinogen (e.g., A) in the blood cell, by the corresponding α agglutinin in the serum: one may then, along with Ehrlich and Morgenroth [25], conceive a mutation-protection, in that an equilibrium is assumed between agglutinogen A which is produced in the tissue cells as well, and the simultaneously produced α in the serum, or else one can postulate the formation of a special protective colloid. The reciprocity which is found in Landsteiner's outline between agglutinogen and agglutinin is only an apparent one, and caused particularly by the recessivity /240 of the R-factor.

5. Concerning the causes of the mutations which have occurred, various hypotheses can be advanced. We should like to draw attention to the fact that the maximum B property incidence is to be found among the rice-eating people of the Orient. It appears likely that the cultivation of rice began after the separation of Indians and Mongols. Whether the food itself or whether injuries of a direct or parasitic kind occurred from its use (and the defense phenomena thereby created within the organism) have caused the mutation, must remain a matter of doubt, particularly since a similar kind of explanation of the A mutation appears to be far-fetched.

6. A firm result for the general theory of evolution is established from points 2 and 3 above in connection with Verzár's observations, namely, that positive mutations, in the sense of the de Vries-Morgan concept, which have arisen at some time or other and in a specific locality and then, in effect, have remained unchanged for hundreds of years, have been unquestionably demonstrated.

I. THE HYPOTHESIS CONCERNING GENES

Along with the hypothesis of the independent pairs of characteristics A and B (see Introduction), the hypothesis of three multiple allelomorphs, of the type for which Morgan has found examples particularly in the Drosophila [8], must be drawn into the picture because similar genetic phenomena arise from it. It should first be mentioned that the hypothesis of multiple allelomorphs corroborates the assumption of the mitotic phenomena in familial heredity, i.e., it excludes possibilities which can arise in terms of the other hypothesis, while on the other hand, all the cases of mitosis occurring under it do not contradict the other hypothesis of independent pairs of genes. The hypothesis of multiple allelomorphs does not deal with hereditary factors which are located either on different chromosomes or on the same chromosome, but in different locations (as is the case with independently "Mendelizing" pairs of genes). It rather deals with hereditary factors which are situated in exactly corresponding locations of one chromosome or its partner. When we start with an individual AA, then in the usual case the formation of multiple allelomorphs means that mutations cause new genes BB, CC, etc., to be generated in the same location. By interbreeding mutated individuals with the original kind there will obviously result individuals with the formulas AB, AC...; and through further interbreeding of the mutants

among themselves, BC... In cases of this kind it is said that the genes /241
A, B and C form a series of multiple allelomorphs. In the present case we shall therefore assume, without first presenting any hypothesis concerning the mutation processes, that there are three multiple allelomorphs, A, B and R, of which A and B are dominant with respect to R, while the heterozygote AB remains differentiable. We therefore have the following figure:

FIGURE III

Classes:	0	B	A	AB
Individuals:	RR	RB BB	RA AA	AB

As comparison of this Figure with Figure II of the introduction will show, a difference is to be expected only in interbreedings in which the parent is an AB individual, and furthermore, the probability of an AB offspring is different in certain matings. Thus, the material is relatively weaker with respect to matings with AB parents or children among Europeans and Americans because of the rarity of the B-factor. It is more frequent in Eastern Asia. Among 72 families with 348 members investigated by v. Dungern and Hirszfeld [4], 1910, the hypothesis of multiple allelomorphs was contradicted by four matings, which supported instead the hypothesis of two independent pairs of genes. On the other hand, the data for the 67 families with 255 members which were investigated by Ottenberg [9] in 1927, are compatible with both genetic hypotheses. In a study published by Jerrell [10] in 1923, using 32 families with 136 members for investigative material and which reports 9 marriages of AB parents which produced AB children, no contradiction of the hypothesis of multiple allelomorphs can be found. In a study by S. Kirihara [11], 1924, in which 139 fami-

lies with 611 persons were observed, there were two items which contradicted the hypothesis of multiple allelomorphs. All told, in 310 families with 1,350 persons, there were contradictions relating to 11 persons from 6 families. A more precise discussion concerning possible explanations of these deviations will be found in the section headed Exkurs 1. The reasons which, in our opinion, /242 speak decisively in favor of the multiple allelomorph hypothesis may be seen from the distribution of the group ratios of the various races. Verification of whether the familial observations fulfill the Mendelian laws was conducted by means of a method different from that employed by v. Dungern and Hirszfeld and subsequently by Ottenberg, in order to isolate the original Mendelian ratios from the racial class-distribution and thus be able to combine the experiences for all of the families so far investigated (see Exkurs, 1).

II. THE GROUP RATIOS TO BE EXPECTED FROM THE TWO HYPOTHESES CONCERNING GENES

The two hypotheses concerning genes yield different expectations with respect to the group ratios in populations, and these differences become appreciable even when the number of observations is not very great. According to the hypothesis of independent pairs of genes, which forms the basis of Hirszfeld's calculations, the following equation is valid:

$$(\overline{A} + \overline{AB}) \cdot (\overline{B} + \overline{AB}) = \overline{AB} \qquad (1)$$

If we use p to designate the frequency of gene A in a closed population, so that $(1 - p) = \bar{p}$ represents the frequency of a, q represents the frequency of B, so that $(1 - q) = \bar{q}$ represents the frequency of b, and if genes A and B are independent of each other, then the probability for the various groups, according to this hypothesis, can be seen in the following table.

Class	Type	Probability
O	aa bb	$(1 - p)^2(1 - q)^2 = \overline{p^2} \cdot \overline{q^2}$
B	aa BB	$(1 - p)^2q^2$
	aa Bb	$2(1 - p)^2q(1 - q)$ $= \overline{p^2} \cdot (1 - \overline{q^2})$
A	AA bb	$p^2(1 - q)^2$
	Aa bb	$2p(1 - p)(1 - q)^2$ $= (1 - \overline{p^2})\overline{q^2}$
AB	AA BB	$p^2 \cdot q^2$
	Aa BB	$2 \cdot p(1 - p)q^2$
	AA Bb	$2p^2q(1 - q)$ $= (1 - \overline{p^2})(1 - \overline{q^2})$
	Aa Bb	$2p(1 - p) \cdot 2q(1 - q)$

This yields the following relationship:

$$\overline{O} \cdot \overline{AB} = \overline{A} \cdot \overline{B}$$

further, because

$$\overline{A} + \overline{AB} = 1 - \overline{p^2}$$

$$\overline{B} + \overline{AB} = 1 - \overline{q^2}$$

we arrive at Equation (1) above:

$$(\overline{A} + \overline{AB}) \cdot (\overline{B} + \overline{AB}) = \overline{AB}.$$

But according to all observations, $(\overline{A} + \overline{AB}) \cdot (\overline{B} + \overline{AB}) > \overline{AB}$, or $\overline{O} \cdot \overline{AB} < \overline{A}\ \overline{B}$. Given a large number of observations, we may exclude the possibility that this inequality could occur by chance. Therefore, I first sought for an explanation of this circumstance in the possibility that the observed population groups did

not constitute a truly homogeneous mixture, and as a matter of fact, if we assume that the magnitudes $\bar{p}$ and $\bar{q}$ represent only mean values for the population of a country, a deviation in the indicated sense can occur (see Exkurs, 2). But the possibility that this explanation is valid is completely negated by the investigations of F. Verzár and O. Weszeczky [7] of the German colonists in Hungary. These colonists, who form a connected population in a few villages, show the same group percentages as the population in their country of origin (Germany) from which they had emigrated 200 years earlier. Non-homogeneity of the distribution, of the kind that can be presumed to be present in the population of a large territory cannot be employed for explanatory purposes here, in view of the small number and the interrelations of the population. In the same sense, but of lesser probative value are the results for the gypsy colonies which, according to the same investigation, were found to be completely identical with the results obtained by Hirszfeld [6] for the Indians.

Equation (1) is even less satisfactory for the East Asian populations, /244 which have been studied carefully and in great numbers (see table). For example, according to Kirihara [11] the following figures apply to the Japanese in Korea (502 persons): $\bar{A} + \overline{AB} = 50\% = 0.500$, $\bar{B} + \overline{AB} = 28.4\% = 0.284$, $(\bar{A} + \overline{AB}) \cdot (\bar{B} + \overline{AB}) = 0.142$, whereas $\overline{AB} = 7.8\% = 0.078$. If the population in this case is to be considered as a mixture with variable $\bar{p}$ and $\bar{q}$ values, then, on the assumption of two components, there will be extreme differences between them which is impossible considering the total geographic results. Thus, the explanation of the deviations from the values required by Equation (1) on the basis of a mixture of populations fails completely (see Exkurs 2).

It definitely follows from these observations that the hypothesis of two independent pairs of genes is untenable. To assume a dependence between the

two pairs of genes, i.e., intercoupling, will not lead to an explanation, since the coupling will be without effect on the distribution in the populations when it is a question of anything other than absolute coupling, i.e., identity. That this must be so will be proven by us in another place, and will only be made plausible here through a comparison*. Although the hypothesis of two genes located at different points in the chromosomes does not lead to agreement with observations, the special hypothesis of two mutated genes A and B and a prototype gene R, which we believe is located precisely at the same spot of a given pair of chromosomes, does yield a relationship between the observed classes which is fulfilled with the definiteness one would expect. If we again designate with the letter p the probability of gene A, and with q the probability of gene B, and with r the probability of gene R in a uniformly mixed part of /245 the population, and that

$$p + q + r = 1, \qquad (2)$$

holds, then we have, for the six Mendelian types:

RR	BR	BB	AR	AA	AB
r^2	$2qr$	q^2	$2pr$	p^2	$2pq$

the respective probabilities

*Imagine a ballroom with a number of women and men, whose powers of attraction towards each other are entirely equal. There is only the peculiarity, which corresponds to the coupling hypothesis, that a man, while he is dancing with a woman, has a greater inclination to dance the next dance with her than to dance with some other woman. On the other hand, the women possess the capacity of making their current partner forget completely with whom he had danced before, so that while he prefers his current partner, he has no particular preference between any of the others. In that case, regardless of the magnitude of the attraction, provided it is not infinite, and assuming that the ball lasts long enough, then every man will come together equally often with every woman, exactly as though his momentary partner did not have a definite power to attract. The only difference would be the amount of time required. Since centuries are available in this case, time plays no role.

and hence for the four classes:

$$\overline{O} = RR \quad \overline{B} = BR + BB \quad \overline{A} = AR + AA \quad \overline{AB} = AB$$

we have the respective probabilities

$$r^2 \qquad 2qr + q^2 \qquad 2pr + p^2 \qquad 2pq$$

Hence we have:

$$\overline{O} + \overline{A} = (r + p)^2$$

$$\overline{O} + \overline{B} = (r + q)^2, \text{ also}$$

$$q = 1 - \sqrt{\overline{O} + \overline{A}}$$

$$p + 1 - \sqrt{\overline{O} + \overline{B}}$$

$$r = \sqrt{\overline{O}}$$

from which we obtain:

$$1 = p + q + r = 1 - \sqrt{\overline{O} + \overline{B}} + 1 - \sqrt{\overline{O} + \overline{A}} + \sqrt{\overline{O}}$$

This equation is not a trivial relation between the observed classes which must always hold, since three percentages, entirely independent of each other, are expressed in terms of only two independent frequencies p and q*.

---

* Under the hypothesis of pairs of independent genes, p and q are determined in the same way. The difference between the two is only in

$$(\overline{A} + \overline{AB}) \cdot (\overline{B} + \overline{AB}) = \overline{AB};$$

where in fact we have

$$p = 1 - \sqrt{\overline{p^2}} = 1 - \sqrt{\overline{p^2 q^2} + \overline{p^2}(1 - \overline{q^2})} = 1 - \sqrt{\overline{O} + \overline{B}}$$

whence we have

$$q = 1 - \sqrt{\overline{q^2}} = 1 - \sqrt{\overline{O} + \overline{A}}$$

Admittedly, by using $\overline{O} \cdot \overline{AB} = \overline{A} \cdot \overline{B}$, which is not fulfilled by actual observation, it becomes possible to transform the above expressions into other formulas which agree with the hypothesis of independent genes, but which do not yield reliable formulas.

The calculated figures p, q, and r possess the following profound significance from a Mendelian point of view. When one considers the population at any location as having arisen from a mixture of pure races then we have to concern ourselves with three pure races, whose Mendelian formulas are AA, BB and RR. These three races have the frequencies p, q, and r. These are the frequencies which must have prevailed at the time that the pure races were in existence, when it is intended to represent the current distribution through a complex mixture of these at any place whatever. We shall designate these as the current mixture ratios of the serological races. From an evolutionary point of view, these races are to be considered in the present case as permanent mutations which presumably, because of the predominance of r, have originated from RR. While in the case of Morgan's Drosophila mutations [8], this permanence may still appear questionable, in this case it has been positively established that mutations of genetic arrangements, once they have occurred, will be maintained for many centuries even through mixtures and back crosses. /246

We shall therefore present the figures for p, q, and r, for all of the populations observed to date, indicating the observer, and with confirmation of the relation: $p + q + r = 1$, in the table on pp. 25 to 29.

The table shows that the prototype race amounts to about 60% everywhere with the exception of the original inhabitants of the American continent and the Philippines, where it rises to about 80% to 90%*. The B race is most frequent in India, as Hirszfeld has already noted, and from there it apparently decreases in all directions and, in point of fact, roughly in proportion to the distance, although perhaps declining somewhat more slowly in the northeastern

---

*See also the Addendum appended during the proof-sheet revision.

direction. Hirszfeld has justifiably thought that a connection existed between the Indians and the European peoples. According to our own determinations there would appear to be both an Indo-Germanic as well as a Germano-Indian migration; the visible outcome of these migrations can be found in the spread of the A race during the first, and of the B race during the second.

III. THE SEROLOGICAL FOUNDATIONS OF THE THREE-RACE THEORY

From the standpoint of the three-race theory with the postulated genes A, B and R, we obtain the following six blood classes:

	RR	RA	AA	RB	BB	AB
Class:	0	A		B		AB,

which we have placed into recognizable relations with the observed four /247 classes. If we designate the agglutinins with the symbols α, β and ρ, and retain for the agglutinogens the same designations as for the genetic factors, then in terms of our concept each of the six classes α, β and ρ will be present in the serum, with the individual characteristic that every time the agglutinin which corresponds to a given agglutinogen is made ineffective by some process, we will indicate this by parentheses. In addition, R and ρ have a particular position, in the sense that, because of the recessiveness of the genetic factor R on the one hand, and of so-far unknown causes on the other, it has not yet been proven that there is a direct action on the part of these probably existing groups. We shall indicate this by the symbol "^". We thus obtain the following schematic representation:

Class:	0	A		B		AB
Formula:	$\hat{R}\hat{R}$	$\hat{R}A$	AA	$\hat{R}B$	BB	AB
	$\alpha\beta(\hat{\rho})$	$(\alpha)\beta(\hat{\rho})$	$(\alpha)\beta\hat{\rho}$	$\alpha(\beta)(\hat{\rho})$	$\alpha(\beta)\hat{\rho}$	$(\alpha)(\beta)\hat{\rho}$

The Landsteiner diagram can be derived from the above by omitting the letters in

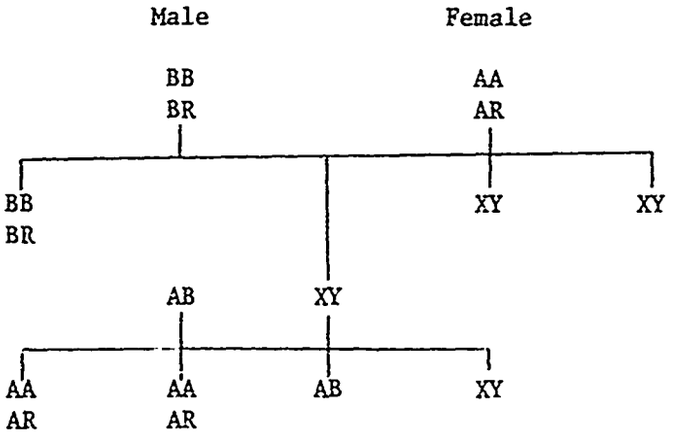
parentheses and the recessive-inactive ρ. The roles of the agglutinins and agglutinogens cannot be interchanged in hereditary phenomena. In fact, the O-class with the agglutinins α and β is homozygous, the AB class which apparently has no agglutinin is heterozygous, i.e., through propagation within the AB class alone, the agglutinins must be present in the AA and BB offspring while being absent in the parents. On the other hand, agglutinogens appear in the children only when they are present in the parents. One can even go so far as to say that the agglutinogens are hence similar to secretions of the chromomeres. The mutation which has given rise to the three races is therefore primarily one affecting the agglutinogens. There is no necessity to assume a priori that the serum was different in the three races.

The fact that autoagglutination does not normally occur may be explained in terms of a process which acts prophylactically. According to Ehrlich and Morgenroth [25] this protection is explainable either through the absence of the relevant group or through a diversion process, and in this case it is only the latter possibility that can be considered. We must therefore assume, in accordance with the lateral-chain theory that when, for instance, an α-agglutinin is produced in the tissue cells of an AA individual, sufficient A ag- /248
glutinogen is supplementarily produced so that an equilibrated reciprocal bonding process arises which protects the red blood corpuscles. This concept will explain why, when repeated transfusions are performed, and even when blood of the same kind is used, autoagglutinations with severe consequences can develop. These are to be considered as having been caused by a disturbance in this prophylactic diversion process. It will also be understood from the point of view of this explanation, why, when the agglutinins first appear in the serum, which occurs in the first months of the individual's life and follows the appearance

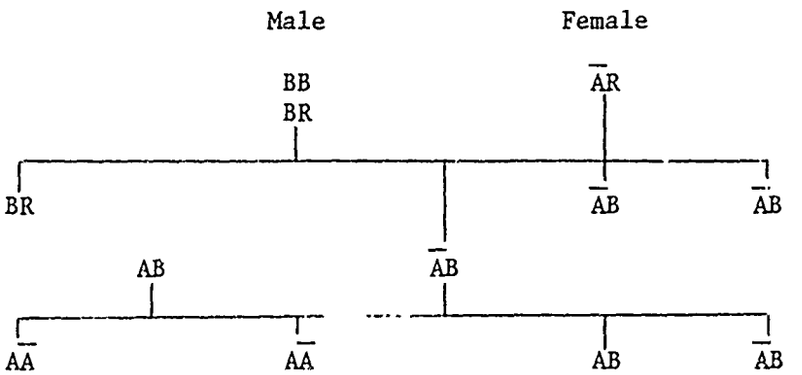
of agglutinogens, spontaneous disorders of this internal regulatory process apparently can arise. It is in fact notably that when the times coincide where agglutinins begin to be secreted and convulsions during childhood appear, it appears to be important to know how these diseases are distributed among the four blood classes. Other explanations of the protective action through specific protective colloids, mentioned by Zsigmondy, would, in a sense, become conceivable and even preferable if it were possible to discover phenomena which would indicate that there is independent superposition of two or more such protective colloid actions. Because of the possibility of assuming a superposition of the actions which have been discussed here, of the kind which, for example, is absolutely necessary for the cells of the AB class, the chemical explanations so far have been characteristically superior. Whether protective colloids or diversion processes are to be assumed, will have to be decided from the changes in the effective titers of serum mixtures. The recessiveness of the R gene appears to be only a conditional one. In actuality, experiments by Hooker and Anderson [26], who have immunized rabbits with human blood from each of the four classes and who were able, following certain preparations, to obtain specific heteroagglutinins in this way, have taught us that the O group also possesses an agglutinogen with a specific action. Whether this will make it possible to distinguish the AA class from the AR class, is still to b determined.

The observations of Guthrie and Huck [19] and also of Coca and Klein [20] have been interpreted by these authors as a demonstration of the presence of new hereditary factors. A specific individual (CT) with a hereditary anomaly has been observed. If we then designate this unknown quality by XY and then assign to the A and B individuals both possible formulas, the family tree takes

on the following appearance:



The blood corpuscles of XY always react as though they belong to the B class, and the serum more frequently acts as though it belongs to the AB class, and less frequently as though belonging to the B class. Hence it is certain that B is a normal, paternally inherited genetic factor. The abnormal factor is derived from the mother. It appears doubtful whether the abnormal factor is A or R. The family tree thus teaches us first of all that the mother is AR when there is a normal B and hence BR child to which she transmits the likewise normal R gene. Therefore it is the maternal A gene that is the abnormal one, and the family tree has the following appearance, if we designate this gene with the symbol $\overline{A}$:



Since the $\overline{A}$ factor seems never to produce agglutinogen, it must be a weak one.

However, it appears to have combined with α agglutinin to some degree. It may be noted, however, that foreign A blood corpuscles produced agglutination in approximately a 13:43 ratio. It therefore seems likely that the agglutinin /250 which remained available was sufficient for the ca. 13 AA's, but was not sufficient for the ca. 43 AR individuals. The case was one of an hereditary partial injury to an hereditary factor A.

Additional experiments, conducted by Guthrie and Huck, were concerned with exhaustion of the agglutinin content of the serum of a person X, for instance from class O, through the blood corpuscles of a second person Y, for instance from class A. It was found that class A was separable into two components A_1 and A_2, so that exhaustion of A_1 still permitted agglutination with A_2. Presumably the formula for the A_1 class was AR, and for the A_2 class the formula was AA. Of course not all X's gave rise to this phenomenon, and some were exhausted by every A. Presumably these contained less, or else less firmly bound agglutinin. There is no reason for the introduction of new genetic factors. However, a statistical testing of the strengths of the agglutinogens and agglutinins, in part through calculation of titers and in part through exhaustion experiments like those of Guthrie and Huck, would appear to be rewarding. Kirihara [11] initiated the first approach with respect to Koreans and Japanese, but unfortunately has presented only average figures. In terms of Goldschmidt's [11] concepts, these variations may be directly related to the genetic factors. Genetic observations of the titer should shed light on this subject.

IV. STATISTICAL COMPARABILITY OF MASS OBSERVATIONS

The statistical comparability of mass observations depends on the agreement of the two test sera A and B. This agreement appears to have been ful-

filled for the investigations by L. and H. Hirszfeld [6] since all of the populations observed by them were tested with sera of Serbian origin. F. Verzár [7] compared his serum with the Hirszfeld serum. The east Asian and the American studies are not a continuation of the ones mentioned first. An apparent continuity between American and Chinese investigations has been achieved by the testing of 111 Chinese students in America, yet the percentages of the A and B classes (29% and 32% respectively) differ so little that true continuity with observations where they deviate strongly from each other is not assured. The observations of Kilgore and Liu [23] with the figures of A = 36%, B = 25% and those of Liu and Wang [24] with the values A = 25%, B = 34%, are subject to the objection that there may have been an interchange of A and B. Since the original work is not available to us, we were unable to substantiate whether a guarantee against interchange had been given. We assume that the determinations of Liu and Wang (1,000 persons) are correct for China. The studies of Fukamachi [27] do not form a continuity with the European investigations, but apparently do with earlier investigations by Hara and Kobayashi [28] and Matubara [29], which unfortunately were not available to us. It is never mentioned if Hara and Kobayashi, or Matubara, ever compared their test sera with European sera. Nor does Fukamachi state, although his study specifically relates to the racial index, whether his test sera had been standardized against European sera. Thus, the question of continuity remains an open one.

V. ISCHEMIA AGGLUTINATION PHENOMENA IN THE HORSE

In a recent study of horses, Hirszfeld has discovered three classes which correspond to the O, A and B classes. For 45 horses he found:

Class	O	A	B	AB
Percent	30	55	15	-

Hence the following figures hold:

$$p = 1 - \overline{B} + \overline{O} = 33\%$$

$$q = 1 - \overline{A} + \overline{O} = 7.8\%$$

$$r = \overline{O} = 54.8\%, \text{ also}$$

$$p + q + r = 95.6\%.$$

Considering the small number of cases the agreement is very good, and here too, would seem to support the concept of multiple allelomorphs, whereas the hypothesis of independent genes which would give a figure of 8.25% for O, is, as always, very inaccurate.

EXKURS 1. VERIFICATION OF THE MENDELIAN LAWS

In verifying the Mendelian laws, the current state of research no longer will deal with the question of testing the Mendelian laws as such on the basis of experiments, since these have been validated by means of investigations running into many thousands of cases, according to the most stringent probability theory criteria. (see, for example, the dissertation of Martin Gauger*, conducted under my direction, in which Lexis' distribution theory was applied to botanical material.) Validation today can only take the form of starting with a genetic hypothesis and then verifying whether the observations agree, within the limits of error which are to be established beforehand, with the a priori predictable numerical ratios. If such agreement is not found, then either the distribution of classes has been shifted on the basis of epigenetic factors, as Correns [12] and Goldschmidt [13] have shown to occur in important cases in their fundamental studies, or else the genetic hypothesis which forms the basis

---

*"The Mendelian numerical series in monohybrids in the light of distribution theory". Göttingen, 1917.

of the study is unsuitable. What we shall test here, therefore, is only the relation of the observed figures to the numerical relations which are yielded on the assumed validity for Mendel's laws and the genetic hypothesis which we have taken as a basis.

We again start with the six formulas: RR, BR, BB, AR, AA and AB. We begin with those matings in which one of the parents belongs to class R and therefore definitely carries the RR formula, while the other parent possesses property A, hence has the formula AA or else AR. The sex cells of the first parent all have the R formula, and those of the second parent are either A or R, hence the offspring is either AR or RR. In the v. Dungern and Hirszfeld [4] observations there are 30 marriages of this type, and the progeny are found to be exclusively AR in 12 marriages, to which v. Dungern and Hirszfeld [4] have assigned the numbers 4, 9, 14, 18, 19, 25, 29, 30, 43, 61, 65 and 68. Where RR children are born of these marriages, we are certain that the type of marriage is RR x AR, since an R has been obtained from each of the parents. In this case, theoretically equal numbers of AR and RR type children are to be expected. The distribution of offspring is shown on the following page.

The total number of RR children amounted to 24 (marriages No. 42, 54 and 58 each contributing only one RR child), and the total number of AR children was 24, where the theoretically expected ratio was 1:1. To be sure, the selection of marriages is not a purely random one, because no marriages are included in which only AR offspring were born through the effects of chance. If we therefore eliminate those marriages which yielded only RR's, and for which the same probability may be presumed, then the asymmetry in the expected RR:AR is again removed. Therefore, 21 RR's are to be compared with 24 AR's, constituting a deviation from the mean value (22.5) which is well within the ordinary

error limits of ± 3.35.

Number assigned to the RR x AR marriage	Number of children	
	RR	AR
3	2	2
6	1	3
20	3	3
23	1	1
34	3	1
37	2	1
41	2	1
47	1	1
50	1	3
52	1	1
56	1	3
57	1	2
60	1	1
62	1	1
	21	24

/253

The cross-type can also be positively inferred in matings of the type "class A x class B", when RR's are found among the children, for in this case both parents must have carried R, so that the cross is of the form AR x BR. The children can never be AA or BB, since one of the parents definitely does not carry A or B respectively. Therefore, insofar as they belong to the A or the B class, they are always either AR or BR. If, say, a child should be AR, it has obtained the R from the B parent, so that this parent must be a BR. Conversely it follows that when a B child occurs, the A parent has the formula AR. Therefore six of the marriages in the material presented by v. Dungern and Hirszfeld can be unequivocally classified. The type AR x BR is given by:

Number assigned to the marriage	The children are:			
	AB	AR	BR	RR
22	1	2	1	-
33	1	1	1	-
35	-	1	1	-
40	1	2	-	1
67	-	2	1	-
70	-	1	1	-
	3	9	5	1

/254

As can be seen, these marriages yielded 3 AB's, 9 AR's, 5 BR's and 1 RR, whereas the expectation was that all combinations would appear to the same extent. But in the light of the small number of observations, the agreement is still within the simple error limits, yet agreement between theory and observation is not too satisfactory. It should also be added, that in the case considered there is no difference in the expectations derived from the two genetic hypotheses. In the interest of abstract theory, we shall discuss this in more detail. Actually, in those marriages in which the parents do not carry the B gene, even in the case of the hypothesis of two "Mendelizing" pairs of genes only one would come into question, while the other, having the property of recessiveness, will be equally represented in all classes and hence need not be considered. Furthermore, in marriages of the type A class x B class, in which a purely recessive child was born, there must have been, according to the hypothesis of two gene pairs, contribution of an "a" from the A parent, and of a "b" from the B parent, so that the cross would definitely be of the form:

Aa aa

x

bb Bb

Similarly, a child from this cross cannot be homozygous in A or B, since only

one of the parents will carry A or B and each parent will always transmit only one gene of one kind. It further follows, from the existence of an A child, which must also possess bb, that the B parent possesses b, hence is heterozygous, and it equally follows from the presence of a B child that the A parent possess a. Therefore, in those cases in which either purely recessive children are born, or children in the A as well as the B class, the cross type of the parent is

$$\begin{matrix} Aa & aa \\ & \times \\ bb & Bb \end{matrix},$$

when they belong to the A or to the B class. Thus, when we suppress the homozygous recessive components which can only exert a recessive influence, we /255 obviously have the same formal scheme for the expected children and hence the same numerical ratios as in the theory of multiple allelomorphs. Therefore the striking deviation found by us bears no relation to the difference between the genetic hypotheses.

Because of the rarity of the B element in the material presented by v. Dungern and Hirszfeld, those marriages in which a B parent is crossed with an RR (= $\overline{0}$) parent are encountered only three times (Nos. 7, 10, 32). According to the same deliberations that were just made for A, they yield four B children and three 0 children.

As v. Dungern and Hirszfeld have already remarked, the cross of 0 parent x 0 parent produces only 0 children.

A difference between the two genetic hypotheses is created by those marriages in which one of the parents is type AB. The following are four marriages out of nine of this type, where recessive children were produced:

Marriage No.	Type	Children			
		AB	AR	BR	RR
39	AB x RR	-	1	-	1
72	AB x RR	3	-	-	1
46	AB x BR	-	-	-	3
71	AB x BR	1	2	1	1

In the case of Family 46, the a priori expectation of a purely recessive child, according to the hypothesis of two independently "Mendelizing" pairs of genes, is only 3/8, even if the strongest hypothetically permissible parental recessiveness is assumed, so that the occurrence of three recessive children is very striking. If reinvestigation were possible, it would yield different results at a different age phase. Equally interesting would be a reinvestigation of Marriage 72, in which the results are admittedly possible according to the hypothesis of independent pairs of genes, but yet are improbable, because the mating was a pure back cross of the heterozygotes

Aa
Bb,

so that the probability of an AB child is only 1/4, and also 1/4 for an O child, while actually there were three AB children and one O child.

Finally, we shall consider class A x class A marriages, namely: /256

Investigator	Number and nationalities of individuals investigated	Class, in percentages							
		O	B	A	AB	p	q	r	p+q+r
L. and H. Hirszfeld	English	46.4	7.2	43.4	3.0	26.8	5.2	68.1	100.1
	French	43.2	11.2	42.6	3.0	26.2	7.4	65.7	99.3
	Italians	47.2	11.0	38.0	3.8	23.7	7.7	68.7	100.1
	Germans	40.0	12.0	43.0	5.0	27.9	8.9	63.2	100.0
	Austrians	42.0	10.0	40.0	8.0	27.9	9.5	64.8	102.2
	Serbs	38.0	15.6	41.8	4.6	26.8	10.7	61.6	99.1
	Greeks	38.2	16.2	41.6	4.0	26.2	10.7	61.8	98.7
	Bulgarians	39.0	14.2	40.6	6.2	27.1	10.8	62.4	100.3
	Arabs	43.6	19.0	32.4	5.0	20.9	12.9	66.0	99.8
	Turks	36.8	18.6	38.0	6.6	25.6	13.6	60.7	99.9
	Russians	40.7	21.8	31.2	6.3	21.0	15.2	63.8	100.0
	Jews	38.8	23.2	33.0	5.0	21.3	15.3	62.3	98.9
	Madagascarians	45.5	23.7	26.2	4.5	16.8	15.4	67.5	99.7
	Senegalese	43.2	29.2	22.6	5.0	14.9	18.9	65.7	99.5
	Annamites	42.0	28.4	22.4	7.2	16.1	19.8	64.8	100.7
	Indians	31.3	41.2	19.0	8.5	14.9	29.1	55.9	100.9

Investigator		Number and nationalities of individuals investigated	Class, in percentages							
			O	B	A	AB	p	q	r	p+q+r
F. Verzár and O. Weszecsky	224	Hungary: Military personnel from the Theiss plain	33.0	22.8	34.8	9.4	25.3	17.7	57.4	100.4
	783	German personnel from Debreczen	31.8	16.1	39.7	12.4	30.8	15.5	56.4	102.7
	133	Patients in the Debreczen Municipal Hospital	36.1	18.8	35.3	9.8	25.9	15.5	60.0	101.4
	274	Patients in the Debreczen Women's Clinic	25.9	24.1	33.6	16.4	29.3	22.9	50.9	103.1
	86	Children's home Debreczen	27.9	15.1	48.8	8.1	34.4	12.4	52.8	99.6
	1500		31.0	18.8	38.8	12.2	29.4	17.0	55.6	102.0
		Germans from:								
	142	Budakesz	39.4	13.4	42.3	4.9	27.3	9.6	62.7	99.6
	167	Budaors	40.1	10.8	46.7	2.4	28.7	6.9	63.3	98.9
	167	Solymar	42.5	13.8	41.3	2.4	25.0	8.5	65.2	98.7
	476		40.8	12.6	43.5	3.1	26.9	8.2	63.8	98.9
		Gypsies from:								
	49	Forro (near Misdolcz)	18.3	38.7	40.8	2.2	24.5	23.1	42.8	90.4
	64	The Debreczen Prison	46.5	24.0	21.2	4.5	16.1	17.7	68.2	102.0
	75	Hajdu-Boszormeny	34.7	39.9	18.7	6.7	13.6	26.9	58.9	99.4
	64	Puspokladany	32.8	37.5	23.4	6.3	16.1	25.0	57.3	98.4
	133	Hajduhadhaz	33.8	45.9	13.5	6.8	10.8	31.2	58.1	100.1
	385		34.2	38.9	21.1	5.8	14.5	25.6	58.4	98.5

Investigator		Number and nationalities of individuals investigated	Class, in percentages							
			O	B	A	AB	p	q	r	p+q+r
F. Verzár and O. Weszecsky	476	German colonists in Hungary	40.8	12.6	43.5	3.1	26.9	8.2	68.8	98.9
v. Dungern and L. and H. Hirszfeld	500	Germans in Heidelberg	40.0	12.0	43.0	5.0	27.9	8.9	63.2	100.0
F. Verzár and O. Weszecsky	1500	Hungarians in Debreczen	31.0	18.8	38.0	12.2	29.4	17.0	55.6	102.0
Hirszfeld	500	Turks	36.8	18.6	38.0	6.6	25.5	13.5	60.7	99.7
F. Verzár and O. Weszecsky	385	Gypsies in Hungary	34.2	38.9	21.1	5.8	14.5	25.6	58.4	98.5
Hirszfeld	1000	Indians	31.3	41.2	19.0	8.5	15.0	29.1	55.9	100.0
Descastello and Sturli	Cited from Jervell	Germans	42.6	17.4	37.4	2.6	22.4	10.6	65.3	98.3
v. Dungern and Hirszfeld			35.6	12.2	47.6	4.6	30.9	8.8	59.7	99.4
Hektoen			47.0	10.0	34.0	9.0	24.5	10.0	68.6	103.1
Moss		Americans	43.0	7.0	40.0	10.0	29.3	8.9	65.6	103.8
Ottenberg			44.0	12.0	42.0	2.0	25.2	7.8	66.3	98.8
Johannsen		Danes	47.3	12.0	36.7	4.0	23.0	8.3	68.8	100.1
Jervell		Norwegians	35.6	10.3	49.8	4.3	32.3	7.6	59.7	99.6

Investigator		Number and nationalities of individuals investigated	Class, in percentages							
			O	B	A	AB	p	q	r	p+q+r
Fukamachi		Koreans in Seoul								
	121	Men	25.61	22.31	35.53	17.35	30.8	21.7	50.6	103.1
	58	Women	24.13	31.20	36.20	8.62	25.6	22.3	49.1	98.0
	179		24.58	25.13	35.75	14.52	29.5	22.3	49.6	101.4
		From Phyengyang								
	100	Men	26.00	33.00	80.00	11.00	23.2	25.2	51.0	99.4
	84	Women	38.09	21.42	29.76	10.71	22.9	17.7	61.7	102.3
	184		31.52	27.71	29.89	10.86	23.1	21.6	56.1	100.8
	363	Total Koreans	28.09	26.44	32.78	12.67	26.2	22.0	53.0	101.2
		Manchus from Mukden								
	127	Men	26.77	37.79	25.98	9.44	19.7	27.4	51.7	98.8
	72	Women	26.38	38.88	27.77	6.94	19.2	26.4	51.3	96.9
	199		26.63	28.19	26.63	8.54	19.5	27.0	51.5	98.0
Hara and Kobayashi		Japanese from:								
	353	Nagano	24.0	16.0	40.5	20.0	36.8	19.7	49.0	105.5
Mazubara		Sendai	32.5	19.2	37.0	11.3	28.1	16.6	57.0	101.7
Fukamachi	170	Fukuoka	24.1	20.2	45.3	10.5	33.4	16.7	49.1	99.2
			26.8	18.4	40.9	13.9	32.8	17.7	51.8	102.3
		Chinese from:								
Fukamachi	35	Chih Li	37.14	25.71	28.57	8.57	20.8	19.1	60.8	100.7
	45	China	31.11	24.44	37.77	6.66	25.6	17.1	55.7	98.4

Investigator		Number and nationalities of individuals investigated	Class, in percentages							
			O	B	A	AB	p	q	r	p+q+r
Kilgore and Liu	100		28.0	25.0	36.0	11.0	27.2	20.0	52.9	100.1
Liu and Wang	1000	Chinese	30.0	34.0	25.0	10.0	20.0	25.8	54.7	100.5
Coca and Deibert	111		29.0	29.0	32.0	10.0	23.8	21.9	53.8	99.5
	862	Indians	77.7	2.1	20.2	-	10.7	1.1	88.0	99.8
Kirihara	502	Japanese in Korea	29.4	20.6	42.2	7.8	29.3	15 4	54.2	98.9
	354	Koreans in Heihoku	30.5	34.5	27.4	7.6	19.4	23.9	55.2	98.5
	311	Koreans in Keiki	27.3	32.8	32.8	7.1	22.5	22.5	52.2	97.2
	112	Koreans in Chuhoku	17.9	33.7	36.6	12.5	28.2	26.2	42.3	96.7
	171	Koreans in Zennan	19.9	25.7	41.5	12.9	32.5	21.6	44.6	98.7
Cabrela and Wade	231	Philippinos (135 men + 96 women)	64.7	19.6	14.7	1.0	8.2	10.9	80.4	99.5

Marriage Number	With Children in:	
	A Class	O Class
24	3	-
26	5	-
27	3	1
31	1	1
36	5	1
49	5	-
59	-	1
64	6	1
69	3	1

/259

Marriages 27, 31, 36, 59, 64 and 69 must be of the form AR x AR, since they contain purely recessive children. In these marriages the expectation is for three-fourths A class children and one-fourth O class children. But once again the selection is not a random one, but those marriages were omitted in which it so happened that the recessives with an expectation of 1/4 did not occur. If we take for the average number of children, s = 4, then for randomly selected material the expected number of recessive children per marriage would be equal to 1, and hence one would expect six such children in six marriages. In order to derive the expected number in the present material, which has been so selected that all marriages in which no recessive child was produced are not taken into account, we may remark that the probability of the occurrence of α recessive children in a marriage which produced s children is given by

$$(1/4)^{\alpha}(3/4)^{s-\alpha}\frac{s!}{\alpha!(s-\alpha)!} = u(\alpha).$$

In this case

$$\sum_{0}^{s}\alpha u(\alpha) = 1$$

eliminating the case where $\alpha = 0$, the sum of the remaining probabilities is given by

$$\sum_{1}^{s} \alpha \, u(\alpha) = 1 - (3/4)^s.$$

When $\alpha \neq 0$, the probability for α is therefore

$$(1/4)^{\alpha}(3/4)^{s-\alpha} \frac{s!}{\alpha!(s-\alpha)!} \left(1 - (3/4)^s\right)^{-1},$$

since the composition of the cases to which the expression $u(\alpha)$ refers, remains unchanged. Hence for α^o the probability is given by the expression

$$\alpha^o = \sum_{1}^{s} (1/4)^{\alpha}(3/4)^{s-\alpha}\binom{s}{\alpha}\alpha \left(1 - (3/4)^s\right)^{-1}$$

Since $\sum_{1}^{s} \alpha \cdot u(\alpha)$ is identical to $\sum_{0}^{s} \alpha \cdot u(\alpha)$ and in the present case obviously /260

equal to $s/4$, we obtain

$$\alpha^o = \frac{s}{4}\left(1 - (3/4)^s\right)^{-1}$$

For $s = 4$, the mean number of children in the marriages discussed here is 1.4628. Hence, we may expect 8.7768 in six marriages, instead of the six observed.

W. Weinberg [14], who was the first to concern himself with the problems of the kind presented here, has employed a method, his well-known sibling method, to estimate the Mendelian value from actual observations. He has applied this method to Lundborg's material with great success and has proven the applicability of Mendel's laws to man, at a time when they were still controversial. His method will not apply here, since none of the recessive children had siblings. Instead of his method, we used an a priori one which also makes it easy to determine the mean error, since we were not interested in testing

the Mendelian laws, but only the genetic hypotheses, having taken the Mendelian laws of inheritance as a basis, in view of the fact that many proofs of their validity in man have been advanced since that time [15, 16, 17]. The square of the mean error, instead of the usual form

$$m^2 = \frac{p(1-p)}{s} = \frac{pq}{s}$$

takes on the form

$$m^2 = \left(\frac{1}{1-q^s} \frac{pq}{s} - \frac{p^2 q^s}{1-q^s} \right),$$

the derivation of which we shall present elsewhere. Using the values $p = 1/4$, $q = 3/4$ and $s = 4$, we obtain

$$m^2 = 0.471$$
$$m = \pm 0.217$$
$$ms = \pm 0.868.$$

The difference between the expected number per family, namely, 1.4268, and the observed figure, is therefore approximately equal to one-half the mean error.

Ottenberg's data [18] contain 17 class A x class O marriages. In the same way as has been discussed earlier, these produced 9 A children and 14 O children, so that when these data are combined with the corresponding ones from the v. Dungern and Hirszfeld study [4], we obtain 33 A children and 35 O children. Fourteen of the marriages are of the B x O parent type and produced 4 B children and 4 O children, which accidentally corresponds to the expected ratio. In this case, families with one AB parent do not lead to a contradiction of the multiple allelomorph hypothesis. /261

S. Kirihara [21] presents information for 21 families of the A class x O class type. If we again confine ourselves to only those families in which both A and O children were born, we obtain 19 A children and 18 O children, so that

in combination with the previous results we have the ratio A children : O children = 52:53, against a theoretical 1:1 ratio. A corresponding test for the class B x class O marriages yields, according to Kirihara's observation, a ratio of B children to O children of 17:18, so that when all of the observations are combined, a ratio of 25:25 is obtained, compared with a theoretical 1:1 ratio.

The marriages which contradict the theory of the multiple allelomorphs are the two marriages, Nos. 6 and 117, out of the 139 marriages in Kirihara's material. Marriage No. 6 consisted of an A father x AB mother, and an O son, and No. 117 consisted of an O father x AB mother, with one AB son and also one A son, two B sons, and one B daughter. In this highly comprehensive study (139 families with 611 members) with 23 marriages of critical composition and ten marriages with AB children, the contradiction becomes minor if two O persons were to be established as being something else, or if their specificity were latent! Furthermore, F. Jervell [10] has reported on six families with AB parents which yield no contradictions. In addition, AB children were born in another four marriages observed by Jervell, again without leading to any contradiction. Were the independent gene hypothesis correct, then in such an extensive total material concerning critical marriages there would occur, one would expect, more contradictions of the hypothesis of multiple allelomorphs. It is remarkable that as more material was gathered concerning AB parents, particularly in recent times, the contradictions of the hypothesis of multiple allelomorphs do not appear to have increased, but instead appear to have decreased. Furthermore, it should not be forgotten that both test sera introduce an individual factor into the calculations which can make itself felt during an extensive investigation. It is conceivable that a test serum of an individual

in class A will also agglutinate the blood corpuscles of some peculiarly /262
constructed individuals in the A class, and therefore will make it appear as though he belonged to class AB. It is also remarkable that the Serbs in the L. and H. Hirszfeld [6] investigations differed a little from their geographic neighbors, which may be due to the fact that the test sera originated from the Serbs. To be sure the Serbs are also anthropologically isolated since they represent the second maximum in body size in Europe. Ottenberg and Unger have reported [22] that deviations will occasionally occur in sera; it should also be noted that most cases of non-agreement with the hypothesis of multiple allelomorphs are of children said to be members of the O class, and that non-occurrence of a reaction does not necessarily lead to a conclusion that the genetic factor is absent, since cases are known in genetics where the reaction of a genetic factor is suppressed by other kinds of influences.

EXKURS 2.

If we set $\overline{p^2} = x$, and $\overline{q^2} = y$, then the frequencies of the four classes are given as follows by the hypothesis of independent pairs of genes:

Class:	O	B	A	AB
Frequency:	xy	$x(1 - y)$	$(1 - x)y$	$(1 - x)(1 - y)$

Then if x' and y' represent the values for a second population which is mixed with the first one in a 1:3 ratio, we have

Class:	O	B	A
Frequency:	$\frac{xy + cx'y'}{1 + c}$	$\frac{x(1 - y) + cx'(1 - y')}{1 + c}$	$\frac{(1 - x)y + c(1 - x')y'}{1 + c}$

Class:	AB
Frequency:	$\frac{(1 - x)(1 - y) + c(1 - x')(1 - y')}{1 + c}$

for the combined population.

Whence:

$$\overline{O \cdot AB} - \overline{A} \cdot \overline{B} = (\overline{A} + \overline{AB})(\overline{B} + \overline{AB}) - \overline{AB} = \frac{-c}{(1+c)^2}(x - x')(y - y')$$

holds. When relationship

$$1 - \bar{r} = \bar{p} + \bar{q} = \bar{p}' + \bar{q}' = 1 - \bar{r}'$$

holds, i.e., when the probability is constant for the prototype race, while /263 the frequencies of the A and the B races vary, as is usually the case, then by using

$$\bar{p} - \bar{p}' = -(\bar{q} - \bar{q}')$$

we obtain:

$$\overline{O \cdot AB} - \overline{A} \cdot \overline{B} = (\overline{A} + \overline{AB})(\overline{B} + \overline{AB}) - \overline{AB} = \frac{-c}{(1+c)^2}(x - x')(y - y')$$

$$= \frac{-c}{(1+c)^2}(\bar{p}^2 - \bar{p}'^2)(\bar{q} - \bar{q}'^2) = \frac{-c}{(1+c)^2}(\bar{p} - \bar{p}')(\bar{q} - \bar{q}')(\bar{p} + \bar{p}')(\bar{q} + \bar{q}')$$

$$= \frac{+c}{(1+c)^2}(\bar{p} - \bar{p}')^2 (\bar{p} + \bar{p}') (\bar{q} + \bar{q}') > 0.$$

Thus, a possibility that the old calculation of the AB class would be in excess of actual observation has been shown to exist in the above sense; of course, this explanation fails because the difference $\bar{p} - \bar{p}'$ must be too great, for, if we employ Kirihara's [11] figures for 502 Japanese in Korea, which we may consider as mixed, then the value 0.064 is obtained for the left side. If we now assume that the mixture consists of equal parts, and consider the observed $\bar{p}$ and $\bar{q}$ as average, then we have to introduce the figures

$$0.064 = 1/4(\bar{p} - \bar{p}')^2 \cdot 2 \cdot 0.707 \cdot 2 \cdot 0.846$$

so that, from

$$(\bar{p} - \bar{p}')^2 = \frac{0.064}{0.707 \cdot 0.846} = 0.1701$$

$$\bar{p} - \bar{p}' = 0.412,$$

we obtain $\bar{p} - \bar{p}' = 0.412$, implying a difference of 40% in the A race distribution, which is impossible.

REFERENCES

1. Landsteiner, K.: Concerning agglutination phenomena in normal human blood. Wien. Klin. Wschr. No. 46, 1901.

2. Decastello, A. and A. Sturli: München. med. Wochenschr. 49: 1090-1095, 1902.

3. Jansky, J.: Sborn. Klin. v. Praze. 8: 85-139, 1906-1907.

4. v. Dungern and Hirszfeld: Group specific structures of the blood; the inheritance of group specific structures in the human blood, etc. Zeitschr. f. Immunitätsforschung. 1909, 1910, 1911.

5. Ottenberg, R.: Medical-legal application of human blood grouping. J.A.M.A. 77: 9, 1921.

6. Hirszfeld, L. and H. Hirszfeld: Serological difference between the blood of different races. Lancet 2: 675-679, October, 1919.

7. Verzár and Wesczeczky: Race-biological investigations by means of ischemia agglutinins. Biochem. Ztschr. Vol. 126, 1921/22.

8. Morgan, T. H.: The Physical Basis of Heredity. J. B. Lippincott, Philadelphia and London, 1919. 305 pp.

9. Ottenberg, R.: Medical-legal application of human blood grouping. Second Communication. J.A.M.A. 78: 12, 1922.

10. Jervell: The forensic meaning of isoagglutination of the red blood /264 corpuscles in man. Deutsch. Ztschr. f. d. gesamte gerichtl. Med. Vol. 3, No. 1, 1923.

11. Kirihara, Sh.: Ischemagglutination in human blood. Ztschr. f. klin. Med. Vol. 99, No. 3/6, 1924.

12. Correns: The recognition of ordinary Mendelian hybrids. Berlin Session of the Royal Prussian Academy of Science. February 28, 1918.

REFERENCES

13. Goldschmidt: Investigations in the field of geographic variation. Arch. f. mikroskop. Anatomi u. Entwicklungsmechanik. Vol. 101, Nos. 1-3.

14. Weinberg, W.: Contributions to the theory of genetics. Arch. f. Rassen-u. Gesellschaftsbiologie 9: 165, 1912.

Weinberg, W.: Effects of selection in biological-statistical problems. Ibid. 10: 417, 1913.

15. Fischer, E.: The Hybrids of Rohoboth. Jena, Fischer, 1919.

16. Baur-Fischer-Lenz: Human genetics, 1923.

17. Bernstein, F.: The statistics of secondary sex characteristics. Göttingen Nachr., 1923.

18. Ottenberg, R.: see reference 9.

19. Guthrie, C. G. and J. G. Huck: The existence of more than four isoagglutinin groups in human blood. Bull. Johns Hopkins Hosp. 34: 37-48; Ibid. 34: 80-88; Ibid. 35: 128-135, 1923. see reference 20.

20. Coca, A. F. and H. A. Klein: A hitherto underscribed pair of isoagglutination elements in human beings. Journ. of Immunology 8: 477, 1923.

21. Goldschmidt: see reference 13.

22. Unger, L. J.: Precautions necessary in the selection of a donor for blood transfusion. J.A.M.A. 76: 1921. see reference 20.

23. Kilgore, A. R. and J. H. Liu: China. Med. Jour. 32: 21, 1918. cited by Coca and Diebert.

24. Liu, Heng, Wang: The National Med. Jour. 6: 118, 1920. cited by Coca and Diebert.

25. Ehrlich and Morgenroth: Hemolysins. Berlin. Klin. Wschr. p. 453 (particularly p. 457), 1900.

REFERENCES

26. Hooker and Anderson: The specific antigenic properties of the four groups of human erythrocytes. Journ. of Immunology 6: 419, 1921.

27. Fukamachi: The biochemical race-index of Koreans, Manchus and Japanese. Journ. of Immunology 8: 291, 1923.

28. Hara and Kobayashi: The Iji-shimbun. Medical News. Vol. 937, 1916.

29. Matubara: Jap. J. Surg. 21: 443, 1920.

Verzár, F.: New studies of ischemagglutinins. Klin. Wschr. p. 929, 1922.

Ottenberg, R.: J. Exp. Med. 13: 425, 1911. cited by Coca and Klein.

Moss: Bull. Johns Hopkins Hosp. 21: 63, 1910. cited by Fukamachi.

Coca, A. F. and O. Diebert: A study of the occurrence of the blood groups among American Indians. Journ. of Immunology 8: 487, 1923.

Cabrela and Wade: Isoagglutination group percentages in Phillipino bloods. J.A.M.A. Vol. 77, No. 20, 1921. cited by Kirihara.

ADDENDUM

(Submitted during correction of proofs)

Since the current paper was written, still further determinations have been published, which we have summarized in the following table:

Investigator		Number and nationalities of individuals investigated	Class, in percentages							
			O	B	A	AB	p	q	r	p+q+r
Jonsson	800	Icelanders	55.7	9.6	32.1	2.6	19.2	6.4	74.6	100.2
Manuila and Popovicu	1594	Rumanians	35.5	14.8	42.0	7.7	28.9	12.1	59.5	100.5
"	461	Slovaks	44.7	15.8	31.3	8.2	22.3	12.9	66.8	102.0
"	400	Little Russians	18.0	22.5	39.2	20.3	36.4	24.4	42.4	103.2
"	688	Transylvania-Hungarians	27.8	20.2	40.8	11.2	30.7	17.2	52.7	100.8
"	301	Transylvania-Germans	33.5	12.0	50.5	4.0	32.6	8.5	57.9	99.0
"	414	Germans	40.0	14.0	42.1	3.9	26.5	9.4	63.3	99.2
"	372	Rumanian Bulgars	31.5	14.8	45.4	8.3	32.0	12.4	56.1	100.5
"	211	Rumanian Jews	26.1	19.8	38.8	15.3	32.4	19.5	51.0	102.9
Schiff	230	Berlin Jews	42.1	11.9	41.1	4.9	26.6	8.8	64.9	100.3
Schiff	750	Berlin Gentiles	37.8	16.4	39.4	6.4	26.4	12.1	61.5	100.0
Lewis and Henderson	270	American Negroes	49.0	18.4	26.9	5.9	18.0	13.0	70.0	101.0

Investigator		Number and nationalities of individuals investigated	Class, in percentages							
			O	B	A	AB	p	q	r	p+q+r
Mino	1391	Italians	35.9	8.6	51.1	4.2	33.3	6.9	59.9	100.1
Tebbutt and Connel	405	White Australians	51.4	7.9	36.0	4.7	23.1	6.6	71.6	101.3
"	141	Australians	57.0	3.0	38.5	1.5	22.6	2.3	75.5	100.4
"	1176	Australians	52.6	8.5	36.9	2.0	21.8	5.4	72.5	99.7
Alexander	225	Scotts	43.6	16.9	33.9	5.7	22.3	12.1	66.0	100.4
Dyke	72	Scotts	42.7	10.7	40.0	6.6	27.0	9.1	65.3	101.4
Sandfort	3000	Americans	44.1	8.3	42.3	4.5	27.6	7.2	66.4	101.2
Culpepper-Ableson	5000	Americans	44.5	14.2	36.1	5.2	23.5	10.5	66.6	100.6
Schütz	253	Englishmen	39.5	7.5	50.6	2.4	31.5	5.1	62.8	99.4
Schütz	930	Englishmen	43.2	11.8	42.2	2.8	25.9	7.6	65.7	99.2
Steffan	500	German Marines	39.8	14.0	42.8	3.4	26.7	9.2	63.0	98.9
Schütz	142	Academicians	38.0	9.1	49.4	3.5	31.4	6.6	61.6	99.6
Schütz	138	Prisoners	50.0	10.9	34.8	4.4	22.0	8.0	70.6	100.6
Schütz	77	Maasholm children	40.2	26.0	33.8	-	18.7	14.0	63.4	96.1
Schütz	105	Germans	39.0	13.3	43.9	4.8	27.7	9.1	62.4	99.2

Investigator		Number and nationalities of individuals investigated	Class, in percentages							
			O	B	A	AB	p	q	r	p+q+r
Ketterer	170	Peter's Valley	33.2	6.4	54.0	6.4	37.1	6.7	57.6	101.4
Harvey Pirie	250	South Africans	52.0	19.2	27.2	1.6	15.6	11.0	72.1	98.7
Sucker	1000	Germans in Leipzig	34.5	16.5	41.5	7.5	28.6	12.8	58.7	100.1
v. Jeney	1172	Full-blooded Hungarians	22.27	27.39	31.65	18.68	29.6	26.6	47.2	103.4
Halber and Mydlarski	11488	Poles	32.5	20.9	37.6	9.0	26.9	16.3	57.0	100.2
"	818	Polish Jews	33.1	17.4	41.5	8.0	28.9	13.6	58.0	100.5

The basic relation p + q + r = 1 is satisfactorily met everywhere, so /266 that we consider the theory advanced by us as fully confirmed. Investigations of families have been recently reported by Mino [1*], Learmonth [2*] and Buchanan [3*]. Mino's family tests have brought confirmation in our sense, but we shall not enter again into any detailed discussion on the subject. Mino deserves thanks for adding a compilation of those families in whom there also were contradictions with respect to the earlier genetic hypotheses.

Investigator	Family Number	Type	Children				
					Contradictions		
Learmonth	12	0 x 0	1 0		1 A		
Buchanan	3	0 x 0	4 0			2B	
	21	0 x 0	1 0		1 A		
	22	0 x 0	1 0				3 AB
	21	0 x A	1 0	2 A			1 AB
Weszecsky	13	0 x A	1 0			1 B	
	15	0 x B			2 A		
Mino	2	0 x 0	4 0		2 A		
	37	0 x 0	2 0		2 A		
	16	0 x A		1 A		2 B	1 AB
	21	0 x A					1 AB
	28	0 x A		2 A		1 B	1 AB
	75	0 x A	1 0				1 AB

It is remarkable that in all of the formerly reported cases of non-conforming families concordance can be obtained by having <u>one</u> person in each 0 group, who has either no A reaction or no B reaction. This is where the explanation must be sought, namely, in those cases where it cannot be assumed that some of the children were born out of wedlock. In his study appearing in Klin. Wschr. No. 46, 1924, Hirszfeld has assumed the possibility that the A and B genes can be inherited in a partially coupled form, and sought to find an explanation in such terms (e.g., his family 72). On this point it can be said, as we have

already stressed, that a partial coupling is not capable of explaining the class relationships found by us, but always must lead back to the earlier unconfirmed relation:

$$(\overline{A} + \overline{AB}) \cdot (\overline{B} + \overline{AB}) = \overline{AB}.$$

The newly obtained results are of fundamental importance to an anthropologic interpretation. There no longer appears to be any doubt that the A race /267 is present among the Australian Aboringines, since Tebbut and Connel have compared them with Australian Englishmen. It remains urgently desirable to obtain determinations for the South American natives in order to clear up the question of their presumed relationship to the Australians. The concept that Japanese designations A and B are correct in terms of continuity seems now to be more likely. In conformity to the anthropologic hypotheses of Klaatsch, Stratz and others, the A race would in fact appear to signify a common component from Australia up to northern Europe; on the other hand, the B race seems to be chiefly concentrated on the Asian continent. A relation to the Mongolian race would appear to be indicated by the increase of the B race northward. On the other hand, the B race has not only been positively located in India, but also among the gypsies and the Senegalese Negroes. It is, therefore, not too easy to consider it as a component which would directly indicate admixture with the Mongolian race. Of course, it should be noted that the high percentage, namely 19%, which was found among the Senegalese Negroes has not been found among the American Negroes nor among the Madegascarians or the South African natives, so that there still remains this particular problem to be elucidated. Since, in contrast to the A constituent, the B component indicates a more completely closed main mass, and since it agrees in terms of its main extension with the north-south course of the Mongolian propagation and then continues it southwards, it

seems proper to assume that the development of the B race took place in northern and central Asia and that it included both Mongolian and non-Mongolian components; the southward spread of the non-Mongolian constituents represents infiltration either directly or through India into the European-Australian part of humanity, on the one hand, and extension from India through Madagascar to Africa on the other. The low B values among the North American Indians, in whom reference to the eyelid folds and the shape of the hair leads to the assumption that they contain a considerable Mongolian component, leads to the conclusions that the Mongolian tribes (of the north) also remained free of the B mutation. Speaking in broad terms, the distribution picture of the A and B race agrees well with the concepts which have been advanced from an anthropological standpoint concerning the migrations of humanity.

Certain individual details in Europe, and particularly in Germany, are of special interest. There is no contradiction implied in the fact that occasionally equalities arise in individual classes where anthropologic differences undoubtedly exist. /268 Thus, Schiff found the same percentages for the A class among the Berlin Jewish and non-Jewish populations. How such equalities can be explained will be clarified by me through a brief and purely theoretical discussion of assumptions concerning the compositions of both constituents. Let us assume, in order to be able to perform calculations for an approximate hypothesis. that the Berlin Jews consist of 30% Nordic races, 60% Mediterranean races, and 10% from the southeastern parts, for which we shall use, in approximate terms, the figures for today's Norwegians, Italians, and Rumanian Jews, then we will obtain Schiff's figures. Assuming, on the other side, that the Berlin non-Jews, all of whom belonged to a low social stratum, consisted of a mixture of 50% Nordic and 50% Slavic-Wendic races, we can then arrive at the

figures for the Berlin population by combining the Norwegians and Russians in equal parts.

The figures for Iceland, by comparison to the Norwegian figures, show a considerable decline in the A component, while the B component has declined only slightly. If we assume that the Norwegians have not changed, then one must conclude that the Icelanders represent a mixture of a part of the population that contained far lower A components and also lower B components, hence is almost purely recessive. If a mixture in equal parts is assumed, then we obtain for the hypothetical components: p = 6.1, q = 5.2. A recessive figure, approximately as high as would be required here, is shown only by the North American Indian. We must assume that we are dealing with a primitive racial element of the polar zone in whom A may perhaps be entirely missing. In that case, an admixture of one-third of this primitive race and two-thirds of the Nordic race would suffice. Some basis for an investigation of these questions may perhaps be furnished by the following figures concerning the hair coloring of the 792 Icelanders studied, namely:

Light blond (lyseblond)..... 30.8%
Red haired..... 5.7%
Dark blond (mörkeblond)..... 29.9%
Dark (mörke)..... 33.6%.

It should be stressed that, with the probable assumption of a 1:2 mixture, the primitive race is to be considered as a pure R race. Since historical /269 data relating to the admixture process are lacking (although that may not be too important, since we are probably dealing with a native and an invading race), the possibility must also be considered that, in part at least, the process was a reverse one, namely, that in the interim the Norwegians had been subjected to a mixture which had given them a greater percentage of A. This leads to the

fundamental question of whether the A race is more closely related to the dolichocephalic or the brachycephalic elements of the European population. Hirszfeld has had his students, Halber and Mydlarski, conduct investigations concerning the connection between the blood groups and the cranial index of Polish recruits. A positive, although low correlation was found between the presence of the A group and body size, which in turn is associated with the blond Nordic type (in the investigation of 428 persons from the Wokowysk District). Investigation of 12,306 soldiers gave a maximum of (A + AB) in the Eastern Departments, in the vicinity of Vilna, and in the three departments of Eastern Galicia, which also exhibit maximum blondness.

On the other hand, the maximum p is found in Little Russia. According to the findings of Prozenko (cited by R. Martin: Anthropologie, p. 670), these are brachycranial with an index of 80.5. We must therefore consider the question an open one as to whether the A property predominates among the dolichocephalics or the brachycephalics. It would be desirable to investigate the hyperbrachycephalic Tyrolese, Swiss, and Gascons.

When the total data are reviewed, we find no contradiction of the concept that the current distribution of the A and B components, are explainable in terms of the known migrations and mixings, and are today no longer influenced by diet, climate, or environmental factors. The primitive races, of whom the Vedans, Dravidians, Bushmen and the Akkas have not yet been investigated, appear to lack both A and B almost entirely, so that their original development would appear to have taken place in the more advanced races. A further temporal indication is given by the fact that the North American Indians possess no B, so the origin of B appears to have taken place only after the Americans had been

settled, and in fact, in Central Asia. The greater continuity of the B property furthermore indicates that it developed at a later date.

REFERENCES FOR THE ADDENDUM

1*. Mino, P.: Heredity of blood groups. Estratto dal Policlin. Rome, 1924.

2*. Learmonth, J. R.: The inheritance of specific isoagglutinins in human blood. Journ. of Genetics 10: 141. (cited by Mino)

3*. Buchanan, J. A.: Medical-legal application of the blood groups. J.A.M.A. 78(2): 89, 1922; Ibid. 79(3): 180, 1922. (cited by Mino)

Tebbutt and Connel: Med. Journal of Australia No. 18, February 25, 1922 and 1923.

Dyke, S. C.: The medical-legal importance of the blood groups. Lancet 2: 1271, 1922.

Dyke, S. C. and C. H. Budge: The inheritance of the specific isoagglutinable substances of human red corpuscles. Proc. Roy. Soc. Med. Vol. 15 (Section on Pathology), p. 35, 1923. (cited by Mino)

Pirie, J. R.: The Med. Journal of South Africa 16: 109, 1921. (cited by Hirszfeld)

v. Jancy, A.: Race-biological investigations in Hungary. Deutsch. Med. Wschr. p. 547, 1923.

Halber and Mydiarski: Hirszfeld Numbers. Klin. Wschr. pp. 1180-1184, 1924.

Lattes, L.: Characteristics and Principles of Blood. Messina, 1923. (A German version is to appear soon.)

A CASE OF REMARKABLY WEAK GROUP SPECIFIC REACTIVITY IN AN ADULT

Werner Fischer and Fritz Hahn

Translation of "Über auffallende Schwäche der gruppenspezifischen Reaktionsfähigkeit bei einem Erwachsenen". Zeitschrift für Immunitätsforschung und Experimentelle Therapie 84: 177-188, 1935.

U. S. ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX KENTUCKY 40121

BLANK PAGE

A CASE OF A REMARKABLY WEAK GROUP-SPECIFIC REACTIVITY IN AN ADULT

Werner Fischer and Fritz Hahn

Although there are numerous cases which show a reduced reactivity of */177 the A-characteristic in the human blood group A_2 and especially in blood group A_2B, the behavior of the blood sample described in the following merits special attention. We are dealing with group A blood which drew our attention when, in the course of daily blood group determination, the tested erythrocytes showed especially weak agglutination.

The blood was from a patient in whose history the following should be mentioned: a 33-year-old man who, following angina, fell ill with what appeared to be a septic syndrome accompanied by shivering. Because of thrombosis we undertook ligation and resection of the left jugular vein and then a tonsillectomy on the same side. After that, trypaflavin treatment. Temperature remained subfebrile, not above 38°C. Then a left knee joint puncture revealing an effusion. The content of the extirpated piece of vein was bacteriologically sterile, as were the blood samples, which were tested several times. Wasserman reaction negative; no tubercle bacilli in the sputum or in the knee joint fluid. Continuous moderate leukocytosis of 11,000 to 17,000; no monocytosis. We still suspected a specifically tubercular disease, without being able to prove it diagnostically.

In the course of seven weeks, we had the opportunity to test the blood of the patient several more times, always arriving at the findings described in the following.

---

*/Numbers in the margin indicate pagination of the original foreign text.

A blood group determination showed that the patient belonged to group A, but that his erythrocytes were weakly or not agglutinated at all with B sera, and hardly at all with A-specific ram blood antiserum. /178

The findings were compiled in the following table. This table shows the results of the tests of known 0, A and B erythrocytes and the blood corpuscles in question, the latter being designated A_x in the table, with 3 0, 2 A and 3 B sera, the serum in question and an A-specific ram blood antiserum.

TABLE I.

	Agglutination of the blood corpuscles of:			
	Group 0	Group A	Group B	A_x
with serum group 0	-	+++	++	+++
" " " 0	-	+++	+++	±
" " " 0	-	+++	+++	+++
" " " A_1	-	-	+++	-
" " " A_2	-	-	+++	-
" " " B	-	+++	-	-
" " " B	-	+++	-	-
" " " B	-	+++	-	±
from the patient	-	-	+++	-
ram blood antiserum	-	+++	-	+

The weaker agglutinability of the patient's blood corpuscles is especially pronounced if we use B sera and the ram blood antiserum, whereas the 0 sera, (with one exception) which proved to have a low agglutination titer, agglutinated the blood corpuscles quite well. This result led us to analyze the receptor mechanism and the antibodies of the blood sample as thoroughly as possible, especially since such a finding, which cannot lead to a wrong result if

perfect investigative techniques are used, would demand the greatest possible interest in view of the observed peculiarities.

The receptor investigation revealed that we were dealing with a very weak A-receptor, which was found by quantitative testing with human test sera, A-specific ram blood antisera, and anti-A immune sera from rabbits in agglutination reactions as well as in complement fixation reactions with blood solutions and alcoholic erythrocyte extracts. The weakness of the A characteristic can be especially clearly observed if we compare the agglutinability of the blood corpuscles of the patient in the following table with that of A_2 and A_2B.

TABLE II. /179

Dilutions of B serum (0.2 cc)	Agglutination of the blood corpuscles of:				
	Group A_1	Group A_2	Group A_1B	Group A_2B	A_x
1:1	+++	+++	+++	+	(+)
1:2	+++	+++	+++	(+)	-
1:4	++	++	++	±	-
1:8	+	+	++	-	-
1:16	+	(+)	+	-	-
1:32	(+)	±	+	-	-
1:64	±	-	±	-	-
1:128	-	-	-	-	-

In the experiment shown above, it is remarkable that the blood corpuscles of the patient were agglutinated even less than A_2B erythrocytes, a finding which is unusual inasmuch as in adults the agglutinability of the A_2B blood is usually below that of the A_2 blood, even if we consider a certain area of variation.

In the investigation with A-specific ram blood antiserum, the result indicated the same findings: the agglutinability of the investigated erythrocytes was considerably less than that of A_2 blood, being minimal to such a degree that proof of the A-characteristic, using ram blood antiserum, was almost unsuccessful. A clear distinction of the blood sample from the weakly coagglutinated O and B erythrocytes is impossible, as can be seen from Table III.

TABLE III.

Serum dilutions (0.2 cc)	Agglutinating effect of A-specific ram blood antiserum no. 41 on blood corpuscles of:					
	Group A_1	Group A_1	Group A_2	A_x	Group O	Group B
1:5	+++	+++	+++	++(+)	++(+)	++
1:10	+++	+++	+++	++(+)	++	++
1:20	+++	+++	+++	++	(+)	±
1:40	+++	+++	+++	(+)	-	-
1:80	+++	+++	+++	-	-	-
1:160	+++	+++	++	-	-	-
1:320	++(+)	++(+)	+	-	-	-
1:640	++(+)	+(+)	(+)	-	-	-
1:1280	+(+)	+	-	-	-	-
1:2560	+	-	-	-	-	-
1:5120	-	-	-	-	-	-

These differences between A_1 and A_2 and the investigated blood could also be observed in the agglutination test with anti-A immune serum, which has /180 not yet been described. In this test as well, we could observe weaker agglutination of the erythrocytes as compared to A_1 and A_2 blood. Of course, the differences were less distinct, corresponding to the higher avidity of immune

antibodies, which are less suited for the agglutination distinguishability of A_1 and A_2 than isoagglutinating sera.

As mentioned in the beginning, the erythrocytes in question were agglutinated less by human B sera than by human 0 sera. This finding seemed remarkable, but was confirmed by further tests. In this context, it was shown that if we use 0 and B sera which are equally (or almost equally) strong when compared to A_1 or A_2, the remarkable weakness of the A-characteristic in the investigated blood can be shown more clearly with B serum than with 0 serum*.

The marked weakness of the A-characteristic was also shown during testing of anti-A immune sera with alcohol extracts of blood corpuscles in complement fixation. A similar experiment is described in Table IV.

As can be seen in this Table, even when concentrated extracts of the blood in question are being used, the A-characteristic is detectable to about the same low degree as it is in A_2B blood, however it reacts strongly in A_2 blood.

A similar, weak receptor effect was clearly observed, if we used hemolyzed and isotonic blood solutions (instead of alcohol extracts). If the weaker, immediate function of the A-characteristic of the blood sample in question is unmistakable, as was seen from the investigations described so far, the question as to whether the mode of action of the group specific receptors is within or below the average limits, becomes of great interest.

In comparison with several A blood samples, the testing of the blood of the patient with anti-0 immune sera of rabbits showed no reduction in function

---

*The findings mentioned here, seem to be of interest and ought to be investigated further. Possibly, we must look for an explanation of this observation in differences in avidity between the anti-A of the 0 sera and the anti-A of the B sera, or (which further investigations indicate) in the different heat amplitudes of the antibodies of isoagglutinating sera used.

TABLE IV. /181

Quantities of the anti-A immune serum which has been diluted 10-fold. ccm	Hemolysis of ram blood with amboceptor and complement after its previous treatment with anti-A immune werum and alcoholic human blood corpuscle extracts* of:				
	Group A_1	Group A_2	Group A_1B	Group A_2B	A_x
0.25	0	Spch	0	st	m
0.15	0	0	0	st	m
0.1	0	0	0	k	m
0.05	0	0	0	k	k
0.03	0	0	0	k	k
0.02	0	k	0	k	k
0.01	0	k	0	k	k
0.006	Spch	k	fk	k	k
0.004	k	k	k	k	k

*The extracts used were evaporated on a water bath and then taken up by physiological NaCl solution, corresponding in quantity to the initial volume.

Translator's note: The single-letter abbreviations were undecipherable.

of the specific receptors, neither in agglutination, nor in complement fixation with extracts and blood solutions. In the agglutination test with bovine serum, which after absorption with AB blood had a selective effect on O and A_2 blood corpuscles and with one ram serum (which also had a clear so-called anti-O quota), a similarly strong O component as in the other A_2 erythrocytes was detectable. The group-specific receptors of the patient's blood, therefore, proved their ability to function within normal limits. Anti-M and anti-N sera had the same effect on blood containing both factors as on other MN erythrocytes.

Deliberations which considered a possibly detectable, increased decomposition or an increased elimination of the A substance, as cause for the A-receptor weakness, lead to tests for A content in the serum and sputum. The A-characteristic in the serum could be shown in agglutination-inhibition experiments, if isoagglutinated sera were used; it was found to be present in smaller quantities than in simultaneously tested A_2 serum. Addition of sputum to one anti-A serum produced no inhibition of the ram blood hemolysis; the A-characteristic was thus not eliminated in the sputum. Since we must take the possible presence of a non-eliminator into consideration, (in the sense in which Schiff described it) and since the urine, stool and the knee joint fluid were not investigated, /182 a definite conclusion regarding the eliminated portion could not be made in the present case.

The antigen function of the blood sample in question was further investigated by means of immunization tests. A rabbit, which even before the immunization had an anti-A quota in its serum, and which was subjected to 3 intravenous injections of 1 cc of the patient's purified erythrocytes, delivered a very strong, typical anti-A serum, which also affected ram blood. Using this immune serum, we still could not detect in either direct tests or in binding or cleaving tests, any receptor which definitely belonged to the patient's blood. The differences, as compared to A_2 and A_1, were equally strong when using the serum as they were when we tested it with other anti-A immune sera (see Table IV). Therefore, we were unable to detect a reduction in antigen function of the A-characteristic by means of the immunization experiment, as compared to the extremely weak direct functioning ability*.

---

*This corresponds to the experiences of Klopstock (Zeitschr. f. Immunitätsf. 74: 211, 1932) and one of us (F. Hahn: Zeitschr. f. Immunitätsf. 83:

The serum of the patient was characterized by a very high iso- and hetero-antibody content, whereas, during experiments in the test tube, the blood corpuscle receptors showed an especially weak reactivity. The iso-anti-B of the patient's serum was stronger than all other serum samples, if compared with 10 randomly selected O and A sera. Further, the serum had very effective cold-agglutinins for A erythrocytes, which, at refrigerator temperatures (6 to 8°C), affected only A_1 blood and also A_2 blood at ice bath temperatures. Hetero-antibodies which were especially remarkable are strongly effective hetero-agglutinins* and heterolysins (for ram blood). The ram blood hemolysins were stronger than in 16 other good serum samples used for comparison, and they /183 were distinguished by the fact that they could not be rendered ineffective by inactivation at 60° or even at 65°C. Only one of the other sera showed a certain amount of thermo-stability. We found small quantities of complement-fixing antibodies in the serum in question, i.e., reagents which affect heterogenetic extracts (ram blood and guinea pig kidney extract).

Because of the extremely weak agglutinability of the described blood corpuscles, it was also interesting to test their binding ability and their possible cleavage effect on already-bound antibodies. Repeated binding and cleaving experiments yielded substantially similar results. One of these

---

257, 1934), who, contrary to Akune (Zeitschr. f. Immunitätsf. 73: 75, 1931), were unable to detect quantitative differences of A-characteristic in the immunization effect observed in rabbits.

*An increased content of human sera was observed in ram blood agglutinins in a case of infectious mononucleosis (Paul and Bunnel: American Journal of Med. Sci. 183: 90, 1932). However, as is apparent from the patient's history, our case did not show any monozygotic increase. This corresponds to the experience of v. Moers-Messmer et al., who found in other human sera, in cases where there was no mononucleosis, an increase of the heteroagglutinins as compared to ram blood (see v. Moers-Messmer: Zeitschr. f. Immunitätsf. 82: 203, 1934, and further literature found there). /183

experiments is described below.

Two cc of undiluted, human O serum were digested for ½ hour, at 37°C, with 0.25 cc of erythrocyte sediment from:

a. A_1 blood

b. A_2 blood

c. Blood of our patient (A_x)

After the binding time, the absorbed serum samples were removed, the agglutinated blood corpuscles cleansed three times with ice-cooled NaCl solution at 0°C, the washing solution poured off, and cleavage of the bound antibodies undertaken at 56°C in the usual way, so that the volume of the cleaving solution was the same as the initial volume, i.e., 2 cc.

The agglutinating effect (volume, 0.1 cc) of the:

I. native serum

II. the 3 absorbed serum samples

III. the 3 cleaving solutions, and

IV. the 3 final washing solutions

were tested in comparison with blood corpuscles (0.1 cc of 1% suspensions) of:

α) the A_1 group

β) the A_2 group

γ) the patient's blood (A_x)

δ) the B group.

Agglutination was determined after centrifuging the tubes, and the agglutination strength was calculated in the usual manner.

As can be seen in column IIa of Table V, the quantity of A_1 erythrocytes used is able to completely bind the anti-A of the O serum, whereas the same amount of A_2 erythrocytes (column IIb) leaves a certain amount of anti- /185

TABLE V.

AGGLUTINATING EFFECT OF AN O SERUM

Serum dilutions (0.1)	I. Native: on blood corpuscles from:				II. After absorption by blood corpuscles of:											
					a. group A_1 on blood corps. of:				b. group A_2 on blood corps. of:				c. A_x on blood corps. of:			
	A_1	A_2	A_x	B	A_1	A_2	A_x	B	A_1	A_2	A_x	B	A_1	A_2	A_x	B
1:1	+++	+++	+++	+++	–	–	–	+++	+++	(+)	–	+++	+++	+++	++	+++
1:2	+++	+++	++(+)	+++	–	–	–	+++	++	–	–	+++	+++	+++	+(+)	+++
1:4	+++	+++	++	+++	–	–	–	++(+)	+(+)	–	–	+++	+++	++(+)	+	+++
1:8	+++	+++	++	+++	–	–	–	++(+)	(+)	–	–	+++	+++	++	-	+++
1:16	+++	+++	+	++(+)	–	–	–	++	–	–	–	++(+)	++(+)	++	–	+++
1:32	+++	++(+)	(+)	++	–	–	–	++	–	–	–	+(+)	++	+	–	++
1:64	+++	++	–	(+)	–	–	–	+	–	–	–	(+)	+	–	–	(+)
1:128	++	+(+)	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1:250	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1:500	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
					III. After cleavage of blood corpuscles of a, b and c (above):											
1:1					++	(+)	–	–	+++	+++	+(+)	+(+)	+++	+++	++(+)	+(+)
1:2					+	–	–	–	+++	+++	+	+	+++	+++	++(+)	+(+)
1:4					–	–	–	–	+++	++(+)	–	–	+++	+++	++(+)	±
1:8					–	–	–	–	+++	++	–	–	+++	+++	+	–
1:16					–	–	–	–	++	+(+)	–	–	++(+)	++	(+)	–
1:32					–	–	–	–	+(+)	–	–	–	++(+)	+(+)	–	–
1:64					–	–	–	–	±	–	–	–	+(+)	–	–	–
1:128					–	–	–	–	–	–	–	–	(+)	–	–	–
1:250					–	–	–	–	–	–	–	–	–	–	–	–

IV. The washing solutions did not show any agglutinins for the erythrocytes used.

bodies unbound, to be bound by the A_1 erythrocytes, which are already somewhat more agglutinable than before the binding. However, the binding power of the patient's erythrocytes is even lower; the blood corpuscles of the patient, which are already less agglutinable than A_2, do not even bind themselves completely to the agglutinins directed against the blood, even with identical amounts of erythrocytes and for the same binding time (column IIc), not even to mention the agglutinating serum components of A_2 or A_1. The cleaving solutions, however, show a completely different behavior, even though they were obtained under the same time and temperature conditions and with simultaneous centrifuging. The cleaving solution of the A_1 blood corpuscles, which has bound all present anti-A, contains only very few agglutinins for A_1 (column IIIa). During the cleaving treatment, the A_2 erythrocytes, which actually bound far fewer antibodies, yield an amount of antibodies with much greater effect (column IIIb), and the optimum quantity of antibodies can be regained from the erythrocytes of the patient, which are the least agglutinable and which have the lowest binding capacity. This result is initially surprising because one would assume that of those blood corpuscles which have bound the greatest quantity of antibodies, the optimum quantity of antibodies should be reclaimed during cleavage, and that those erythrocytes which have the lowest binding capacity would only yield a small amount of antibodies. However, this assumption would be correct, if in the subgroups A_1 and A_2 we were merely dealing with quantitative differences in receptor development (of the available quantity of receptors). According to the work of Friedenreich*, Hirszfeld** and Hahn***, one must not only

*Friedenreich, V.: Zeitschr. f. Immunitätsf. 71: 283, 1931.

**Hirszfeld: Weichardts Ergebnisse 15: 75, 1934.

***Hahn, F.: Zeitschr. f. Immunitätsf. 83: 95, 1934.

assume quantitative differences between the subgroups A_1 and A_2, but also dissimilar avidity conditions of the receptors. Thus, the initial, seemingly /186 paradoxical behavior, which is clearly expressed in the binding and cleaving experiments shown in Table V is easily understandable. The anti-A antibody which has a strong affinity (avidity) for A_1, is bound quickly and completely; cleavage of the anti-A, which has bound with strong affinity, is rather insignificant. The anti-A antibody, which has a low affinity to A_2 blood, is less completely and less firmly bound; cleavage of the less firmly bound anti-A yields a greater quantity of reclaimed antibodies. Most successful is the cleavage from the blood corpuscles of the patient which have the lowest agglutinability and which have the lowest binding capacity*.

In the context of the described binding and cleavage experiment, which was repeated several times with other A_1 and A_2 erythrocytes and which had principally the same results, there is one other peculiarity which we have not touched upon. As is evident from columns IIIb and IIIc, we can detect in the cleavage solutions of A_2 erythrocytes and of the patient's blood corpuscles, a small quantity of antibodies for B blood corpuscles. This finding, which was also occasionally made during the cleavage of A_1 erythrocytes, can probably most simply be explained in terms of a small, unspecific absorption, similar to "physiological coupling" (Hirszfeld**) or "secondary binding" (Thomsen and

---

*Similar findings were made by one of us (Fischer) during the binding of ram anti-B immune serum to human B blood and rabbit blood. In this instance, too, cleavage of the rabbit blood which has very low agglutinating power, was successful, with a better yield of antibodies than that of the B blood, which has very high agglutinating power and which absorbed all the antibodies. (W. Fischer: Zeitschr. f. Immunitätsf. 84: 136, 1935).

**Hirszfeld: Ergebn. d. Hyg. 8: 367, 1926.

Worsaae*).

If it is evident from all the tests of the patient's blood that we are dealing with a weak A-receptor, the cause for its low ability to function still remains unexplained. We hope that we will be able to test the blood once again at a later date and to be able to answer the question, whether and to what degree the remarkable weakness of the A-characteristic can be attributed to /187 the sickness. The problem concerning the possible genetic cause of this peculiar blood characteristic could not be investigated, since both parents had died, and since other blood relatives could not be investigated.

Concerning methodology, the behavior of the tested blood affords us another example of the necessity of not being satisfied with testing blood corpuscles in the determination of blood groups, but to always include the serum in the investigations. Further and aside from the anti-A and anti-B test sera, it again demonstrates the necessity to use group O serum, since, as has been seen in this case, insufficiently developed group A receptors can be more easily detected with group O serum than with group B serum.

SUMMARY

1. We are reporting on the blood group analysis of a clinically, still-unsettled case, in which the blood corpuscles distinguished themselves through a definite weakness in the immediately detectable function of the A-receptor. The agglutinability of the blood sample, which belonged to group A, was even lower than that of samples of groups A_2B, which were tested for comparison. One circumstance is remarkable, viz., that this weak A-characteristic could more easily be detected with serum of group O than with serum of group B or

*Thomsen, O. and E. Worsaae: Zeitschr. f. Rassenphysiol. 2: 1, 19, 1929.

with ram blood anti-serum.

2. We could detect an especially high content of iso- and heteroantibodies in the serum, whereas the A-characteristic of the erythrocytes was developed only to a very small extent.

3. Binding and cleavage experiments, including blood samples of group A_1 and A_2, showed that although the binding capacity decreases, corresponding to lower group-specific agglutinability, the cleaving experiments permit us to reclaim more antibodies, the weaker the binding capacity or the agglutinability. Thus, we were successful in reclaiming far greater quantities of group-specific A antibodies during cleavage of A_2 blood corpuscles than during cleavage of A_1 blood corpuscles, however, during cleavage from the tested A blood, which had especially low agglutinating power, the largest amount of antibodies could be reclaimed. /188

4. These investigations indicate, therefore, that the differences in affinity, as expressed in different binding capacities, are also expressed in the firmness of the binding. The less firmly the antibody binding, due to lesser affinity, the higher is the yield of antibodies which can be reclaimed through cleavage.

A SO-FAR UNKNOWN BLOOD GROUP CHARACTERISTIC (A_3)

V. Friedenreich

Translation of "Eine bisher unbekannte Blutgruppeneigenschaft (A_3)". Zeitschrift für Immunitätsforschung und Experimentelle Therapie 89(6): 409-422, 1936.

U. S. ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX KENTUCKY 40121

BLANK PAGE

A SO-FAR UNKNOWN BLOOD GROUP CHARACTERISTIC (A_3)

V. Friedenreich

Investigations in recent years have shown that the original Landsteiner */409 blood group system (ABO system) is composed of 6 classes, since the serological characteristic A includes two variants which are commonly designated A_1 and A_2**. The serological difference between these two receptors is still unsettled, however, it can be roughly expressed by saying that the A_2-receptor, at least in simple serological terms, is characterized by a weaker reaction than A_1.

The original ABO system is therefore comprised of 4 serological groups, viz. O, A, B and AB, of which the second and fourth can again be subdivided into two subgroups. Viewed from a genetic standpoint, we are dealing with 6 groups (phenotypes), since the A_2 characteristic is due to an independent allelomorphic gene, and from the combination possibilities of the four genes (O, A_1, A_2 and B), we find 10 genotypes and 6 phenotypes.

Most recent experience makes it seem highly probable that a differentiation, in principle analogous to the A_1A_2 relation, also exists within the MN blood group system (type N_2) [1].

In a recently published communication [2], I have described the presence of a so-far unknown, apparently very rare, third type of A blood corpuscle, which is distinguishable from the types known in that it reacts much less strongly.

---

*/Numbers in the margin indicate pagination of the original foreign text.

**Concerning the literature, I would like to refer to the handbooks and digests of recent years.

It was shown that: (1) we are dealing with a characteristic serological type, since no transition between the extremely low reactivity of this A-receptor and the A_2-receptor (whose small area of variation has been confirmed by recent investigations [2]) could be found, and because (2) the characteristic is restricted to families. We could not formulate an opinion as to the inheritance of this characteristic on the bases of the two families which were investigated at that time. /410

In the present communication the genetic and most important serological results of the continued investigation of the relatives of the 6 individuals will be discussed. These 6 individuals are independent from each other. The material comprises a total of 260 persons, of whom 49 are of the weak A type. Since, as will be seen, these investigations make it appear quite clear that we are dealing with a third A type of the same series as A_1 and A_2, we will designate the third type as A_3.

It should also be mentioned that according to our serological experience, there is hardly a doubt that in the case described by Fischer and Hahn [4], which was concerned with an unusually weak A-receptor in a sepsis patient, we are dealing with an individual of such a type.

A. BASIC SEROLOGICAL CHARACTERISTICS

1. Agglutination reaction. A main characteristic of these blood corpuscles is that they agglutinate much more weakly than A_2 blood corpuscles, let alone A_1

The difference can be explained in a simple way. It is known that the A_2-receptor of the A_2B group reacts much weaker than the "pure" A_2. According to my experience, this diminution is quite constant, so that the difference

between the reaction of A_1 and A_2 blood corpuscles can be expressed with the words that the latter is weaker, or, at most, as weak as the reaction of the A_2B blood corpuscles*. Table I shows the agglutination reactions of 3 different A_3 individuals, belonging to 3 different families, to 5 anti-A sera of decreasing strength (titer, as compared to A_1 blood corpuscles of above 1000 to 32), when compared to A_2 and A_2B. /411

TABLE I

THREE A_3, AS WELL AS A_2 AND A_2B BLOOD CORPUSCLES, OPPOSITE FIVE ANTI-A SERA OF DECREASING STRENGTH. THE NUMBER OF + INDICATES THE STRENGTH; ± REPRESENTS THE WEAKEST POSITIVE REACTION; [ ] DESIGNATES THE PECULIAR AGGLUTINATION PICTURE DESCRIBED IN THE TEXT. GLASS SLIDE REACTION WAS READ AFTER 15 MINUTES.

Blood corpuscles	B-Sera				
	Z.	BN	J.	Kr.	79
A_2	++++	++++	+++	+++	++
A_2B	++++	+++	++	+	trace
A_3 ("E.")	[+	[+]	[+]	[±]	[±]
A_3 ("W.")	[+]	[+]	[+]	[±]	[±]
A_3 ("L.")	++	+	[+]	[±]	[±]

As can be seen, the two weakest sera give an almost maximum reaction with A_2 blood corpuscles, but hardly any reaction with A_2B and A_3. It is especially

*Hirszfeld [5] has rightly pointed out that this statement is not supported by my own tables [3]. However, this is merely due to the way it is expressed (numerical values for the titer). It is not seldom that one finds rather high titer values during the titration of A_2B blood corpuscles with strong anti-A sera; however, we are dealing here with a series of very weak reactions, whereas the A_2-agglutination in the corresponding dilutions is very strong. Titer results of this kind are hard to render in numerical values.

characteristic that in the stronger sera, the A_3 reaction increases only insignificantly, with the exception of the 3rd person in the extremely strong serum "Z" (see below), whereas the A_2B reaction increases considerably. It must be added that the A_3 reaction, aside from the fact that it is very weak, shows yet another characteristic picture, viz., the slow appearance of a few, not very small, but rather brittle agglutinates, among other complete non-agglutinated blood corpuscles (glass slide reaction; 1 drop of serum + 1 drop of 2-5% blood corpuscle suspension; reading after about 15 minutes. This technique seems to be superior to the test tube or centrifuge method, at least as far as detection of A_3 is concerned).

Figure 1 shows how weak the A_3 reaction is and at the same time demonstrates the practical significance of the A_3 type. The picture shows the result of a sample reaction with 3 different, commercially available anti-A test sera from three different countries, with A_1 and A_3 blood corpuscles. As can be seen, the A_3 blood corpuscles yielded either no reaction or a very slight reaction.

From Table I we can see that the typing of A_3 can be easily performed /412 by comparing the reaction of the blood corpuscles showing A_2 and A_2B reactions, with one or a few suitable B sera. (Such sera can easily be found by screening a small series of B sera*).

The diagnosis is therefore easier than the differentiation between A_1 and A_2, for which purpose the use of absorbed anti-A sera or similar agents is demanded. This can easily be understood, since the "jump" of the agglutinability

---

*More details will be described elsewhere. Here, we will only mention that a complete, or partial lack in ability of a serum to react with A_3, does not seem to be exclusively a function of a low agglutinin titer (e.g., measured by titration with A_1).

Figure 1. Reaction of A_1 and A_3 blood corpuscles with three sample sera. Technique as described above.

(as can be seen from Table II) between A_2 and A_3 is much greater than between A_1 and A_2, where the titer difference is usually only 1 to 2 steps.

TABLE II

TITRATION OF A_2, A_2B, A_3 AND A_3B AGAINST B SERUM OF AVERAGE STRENGTH

Blood corpuscles	B serum, diluted								
	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256
A_2	++++	+++	+++	++	+(+)	+	(+)	trace	0
A_2B	+(+)	+	(+)	trace	0	.	.	.	.
A_3	+	(+)	?	?	0	.	.	.	.
A_3B	0	0	.	.	.	.	.	.	.

2. The absorption capacity of the A_3 blood corpuscles, strangely /413 enough, lies between A_2 and A_2B, i.e., it is stronger than that of A_2B even toward sera which agglutinate A_2B much more than A_3. We must forego an

explanation of this phenomenon for the time being.

3. The α_2-reaction: Reaction of A_3 blood corpuscles with α_2-agglutinin (anti-O). For theoretical reasons, one could hold the opinion that the reaction is stronger than that of A_2 blood corpuscles; in certain cases we did indeed observe a stronger reaction than that of average A_2 blood corpuscles, however, there seems to be no definite regularity. Continued investigations with selected, suitable anti-O sera will be necessary to solve this question, which is quite interesting from a theoretical point of view.

4. The M and N reactions of the A_3 blood corpuscles are entirely normal.

5. The agglutinin content of A_3 sera. For practical application, the investigations had the very important result that in the usual reactions, no anti-A was ever found in the sera of these individuals, in spite of the barely detectable A-receptor. Neither did we find α_1-agglutinin (active at room temperature), and only in a very few cases did we find a small trace of α_1-"cool-agglutinin" (active at 2 to 5°C). The β-agglutinin content of the A_3 sera was in no case different from the usual A sera. The observation of Fischer and Hahn, concerning the agglutinin content of the described person, therefore represents no characteristic peculiarity for the A_3 type (but can possibly be explained in terms of the illness of the patient).

6. The type A_3B. The material includes three cases of this type (belonging to two different families). They seem to be completely similar to each other and show, as is to be expected, an especially weak A characteristic, which can only be detected by very strong sera. The absorption capacity was markedly weaker than in the case of A_2B. In one case, we found an α_1- /41
agglutinin, active at 10°C, with no reaction at room temperature.

7. Concerning the area of variation of the sensitivity of the A_3-receptor,

some observations should be mentioned which are concerned with the investigation results on the inheritance of the described characteristic.

At this time we can make no statement as to the frequency of this trait. The six cases, which formed the starting point of these investigations, were observed within one year in the course of routine blood group determinations, which corresponds to 6 among a total of 4,000 to 5,000 individuals. It is apparent that the A_3 type can very easily be overlooked, if its existence is not known, since its detection depends entirely on the technical circumstances: if very strong sera are used, it can be mistaken for A_2, if the sera are too weak (and if the agglutinin content of the blood sample is not taken into consideration) one will think it is the 0 type.

B. MODE OF INHERITANCE OF THE A_3 CHARACTERISTIC

This is explained in the genetic diagrams 1-6 (Figures 2 to 7).

All blood samples were examined on the day they were taken. The A_3 diagnosis was made after the investigation of the blood corpuscles with a register of B sera, as those described in Table I, simultaneously comparing the sample with standard A_2 and A_2B, and in most cases with A_3 blood corpuscles. Some of the A_3 individuals of each family were also investigated by means of absorption tests. For the differential diagnosis of A_1 and A_2, "α_1" and "α_2" agglutinins were used, i.e., a B serum absorbed by A_2 blood corpuscles, or one or more anti-0-containing bovine sera absorbed by A_1B blood corpuscles. All A_2 individuals were further subjected to absorption tests. All persons were tested as to their M and N characteristics. Further, we tested some "random samples" of A_1 individuals from each family by titration with α_1, and the degree of strength of the M and N characteristics of some, especially of A_3 individuals, was measured.

Disregarding the few exceptional A_2 individuals, mentioned on page 11, all non-A_3 individuals proved to have completely normal group characteristics, even as regards quantitative measurement.

/415

Family 1.

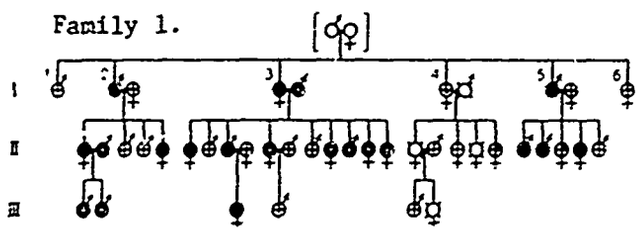


Figure 2.

Family 2.

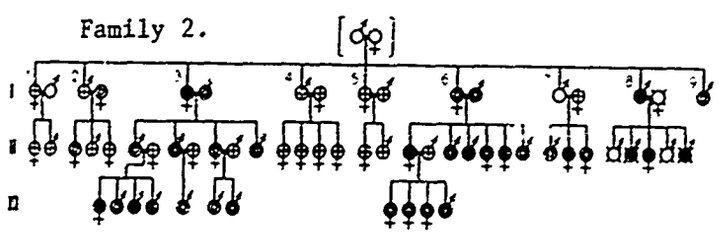


Figure 3.

Family 3.

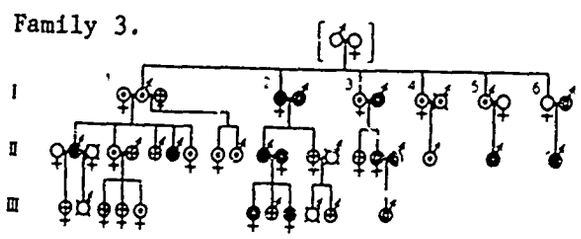


Figure 4.

Family 4.

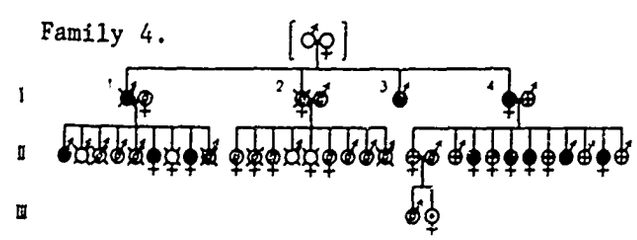


Figure 5.

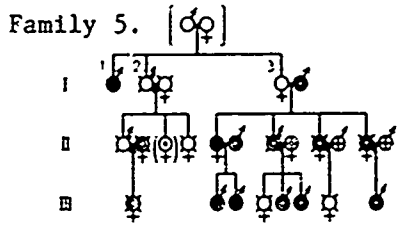


Figure 6.

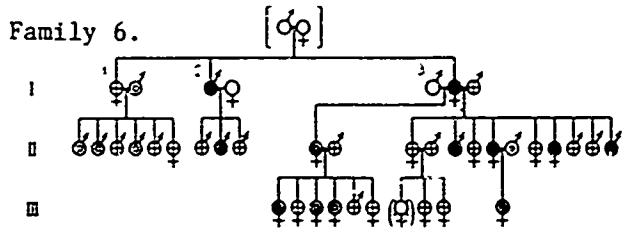


Figure 7.

Legend for Figures 1-7: Familie = Family; Abb. = Figure; ⊖ = group O; ⊖ = group A_1; ⊖ = group A_2; ◑ = group A_3; ⊟ = group B_1; ⊠ = group A_1B; ⊠ = group A_2B; ⊠ = group A_3B; O = dead.

The result of the investigation of the families of these 6 persons, a total of 260 individuals, can be seen from the genetic diagrams (for legend see above).

Summarizing the conclusions from these blood group combinations, we obtain the following results:

1) No A_1 or A_2 descendants from A_3 x O marriages.
2) On the other hand, A_3 descendants do occur in A_1 and A_2 marriages (belonging, of course, tc the "A_3 families").
3) Common A parent with A_3 descendants has no O children (one single exception, see below).
4) No A_3 descendants from marriages between non-A (i.e., O or B) individuals from one of the families and an outside A partner.

In a compilation of those group combinations whose offspring are so numerous that the figures receive a certain significance, we obtain the following picture:

	Total number of children	Group 0	Group A_1	Group A_3
1. $A_3 \times 0$	30	15	-	15
2. $A_3 \times A_1$*	22	4	12	6

i.e., in case one, an exact ratio of 1:1, in case 2 almost exactly 1:2:1.

This mode of inheritance, as well as these numerical relationships, /417 agree accurately with the mode of inheritance of a 3rd allelomorphic A gene, and can probably not be explained in any other way. The A_3 gene must therefore be similar to the genes of the ABO system, however, it is dominated by A_1 and A_2 just as A_2 is dominated by A_1.

The probability of this hypothesis is further increased by the fact that the existence of another gene, whose effect is essentially, or only quantitatively different from the effect of the rest of the A genes, would completely agree with numerous experiences from the animal and plant world.

Thus, the familiar 4-gene system would have to expand to a system consisting of 5 genes, including 15 genotypes and 8 phenotypes:

Genotype	Phenotype	Genotype	Phenotype
00	0	A_3O, (A_3A_3)	A_3
A_1O, A_1A_1, A_1A_2, A_1A_3	A_1	BO, BB	B

*Almost all of these individuals must belong to the genotype A_1O. (This is evident from the fact that they either have 0 children, or are themselves children of 0 parents).

Genotype	Phenotype	Genotype	Phenotype
A_2O, A_2A_2, A_2A_3	A_2	A_1B	A_1B
		A_2B	A_2B
		A_3B	A_3B

Of course, one cannot say whether the genotypes A_3A_3 would be phenotypically identical with A_3O, since individuals of this kind can only occur very rarely.

As said before, our material contained one exception to the rule (family 3), viz., an O son from a parent (I, 1), whose genotype, according to the system, is A_2A_3 and who should therefore be unable to have an O descendant. However, since it is the father who belongs to the A family, we could be dealing with a case of illegitimacy (the material contains 2 cases of proven illegitimacy, which are bracketed in the diagrams; in both cases, we were obviously dealing with very loose marital relations). Certain other possibilities will be discussed at a later occasion; nevertheless, this single case cannot upset the genetic rule, which is otherwise without a gap and which is quite simple.

This family also has another peculiarity worth mentioning, in that it /418 is the only one of all families where group characteristics (other than A_3) were observed which deviated from the norm.

Those individuals which were designated with A_2 in the family tree but which belonged to the A_3 family, possessed the peculiar characteristic that their blood corpuscles gave a very weak α_1 reaction (and therefore had a somewhat higher agglutinin binding capacity than the usual A_2 blood corpuscles). One could therefore consider them to be unusually weak A_1 individuals (the blood group findings of their children did not give us any clues in this regard,

since they had almost all married A_1 individuals). I have preferred to group them with A_2, since there were only traces of the α_1 reaction, and since their α_2 reaction was as strong as that of normal A_2 blood corpuscles.

Only the future will show us whether we are dealing with something other than a chance meeting of two characteristics in one and the same family. It should be mentioned that we are not confronted with an intermediary A type of the kind described by Landsteiner and Levine [6], since the characteristic of the latter is a weak α_2 reaction. Neither can this peculiarity be an expression of the genotype A_2A_3, since it occurs in several individuals of the second generation, who cannot belong to this genotype. Finally, it must be emphasized again that the A_1-receptors of all other investigated families are normal in every respect and that we can, therefore, conclude that the occurrence of unclear A group traits is in no way characteristic for A_3 families. On the contrary, the family under consideration represents an exceptional case.

Let it be mentioned that this family is the one which was investigated first, and whose case had been published earlier [2].

At that time we had to leave the question on the mode of inheritance unanswered, in part because of the above-mentioned deviation from the allelomorph hypothesis, and, in part because all family characteristics (including this case) could be explained in a formally, very simple manner, viz., by the assumption of a dominant, receptor-modifying, "attenuating" gene. It could be imagined that this gene would modify the A_1-receptor to a strength similar to /419 that of A_2, and the A_2-receptor down to the strength of the type here designated with A_3. From a marriage such as $A_2O \times A_1O$ plus this gene (i.e., apparently $A_2 \times A_2$), we could thus expect "A_2" and "A_3", as well as O descendants, as was the case in family 3. However, as is evident from the other family

trees, this hypothesis is completely irreconcilable with the experience gathered so far.

Finally, we will briefly discuss our experience regarding the area of variation of the A_3-receptor. Without doubt, the area of variation is very small, although it cannot be very easily expressed, primarily because there are certain differences among A_3 individuals which apparently must be due to subtle qualitative peculiarities, the nature of which we are definitely familiar. This observation may possibly have certain general biological interest. Thus, for instance, all members of families 2, 5 and 6 react almost exactly as the 3 representatives of Table I. Of the latter, person L is obviously stronger than the others, a fact which, however, only becomes apparent if the strongest sera are used. In this respect, the A_3 members of family 1 are a most curious phenomena; the blood corpuscles of these persons are similarly agglutinated to those of 5, i.e., relatively strong, however, they are distinguished from the other families by the fact that they have a much weaker absorption, even weaker than A_2B (see p. 5). For the time being, we do not have a serological interpretation of this repeated observation.

C. PRACTICAL CONSEQUENCES OF THE EXISTENCE OF THE A_3 TYPE

Since A_3 is completely analogous to A_2, the recognition of the existence of this group characteristic represents an expansion of the ABO system, which theoretically could be applied in the ruling on cases of doubtful parenthood in the same manner as the differentiation of A into A_1 and A_2. However, because of the rarity of this type, this will be more a matter of curious interest. The practical significance of the A_3 group (see p. 3 and Figure 1) is that it can lead to erroneous blood typing, e.g., mistaking A_3 for O and A_3B for B.

Upon closer consideration of the significance of this source of error, two main questions must be asked:

1) How great is the risk that such a mistake could occur? /420

2) What consequences would a possible erroneous diagnosis have?

1. The danger of a wrong diagnosis depends, of course, in part on technical circumstances, primarily on the strength of the test sera used for blood group determination. It seems as if one should make even stricter demands on the strength of the test sera, and in part, on biological considerations, and in this context on the very important question of whether we can be absolutely sure that the serum of A_3 individuals contains no anti-A agglutinin, even though the receptor is extremely weak. When we can generalize this experience, we should be able to avoid incorrect diagnoses.

However, with all due caution we must say that a single observation, which is the subject of a yet-incomplete analysis, does not preclude the possibility that circumstances are not quite as simple as they appear.

2. If, in the future, it should be proven that we cannot absolutely rely on a correct diagnosis by means of serum investigations, then we must consider the consequences of an erroneous diagnosis, partially as they apply to blood transfusions and partially with regard to the legal-medical use of blood typing, especially in cases of doubtful fatherhood.

In blood transfusions, a consequence of such an error could be that an O recipient would receive A_3 blood, instead of O blood and a B patient, A_3B blood instead of B blood. Of course, the consequences of this cannot be foretold, however, we may hope that these blood corpuscles, which react so weakly in vitro would be hemolyzed in vivo to such a small extent that shock could be avoided.

In cases concerning fatherhood, the consequences will depend entirely on whether the genetic hypothesis described above is correct or not.

For instance, if we have the combination O x O with an A (A_1 or A_2) child, we can exclude the fatherhood of the husband, and this exclusion will be correct, even if he (or the mother) have an undetected A_3 trait; because ac- /421 cording to the system, an A_3 individual cannot have common A descendants other than with a common A partner. The same is true for B (i.e., really A_3B) parents with regard to an A child.

The certainty of excluding one type (O child, AB "father") should not be influenced by this: the man must give A (A_1 or A_2) or B to his descendants, so that a person of the usual AB type cannot be the father of that child, even if the child should have an A_3 trait which could possibly be detected at a later time.

However, the following combination merits our special attention: non-A mother x non-A father, with an A child, whose A-receptor is very weak. This child may belong to the A_3 group and one of the parents can be a "latent A_3"*. In other words, we must be able to diagnose A_3, and when it occurs in a child of such combination, we must consider the possibility that one of the parents may have a hidden A_3 trait.

The consequences would be quite different if we were dealing with a different mode of inheritance, as e.g., with a modified gene (see discussion on p. 13). If this would be the case, it would mean that in each exclusion of

---

*The risk would be greatest in those cases where one of the parents is a B individual; it is not unlikely (see p. 6) that in the case of A_3B, the A_3 may be overlooked, so that the A_3B is considered to be B, whereas in the child this characteristic is likely to be detected, since in that case the A_3 trait is not "suppressed" by B.

fatherhood which is based on the presence of an A characteristic in the child, we would have to exercise a certain amount of caution.

SUMMARY

1) Six cases of unusually weak A-receptors were found during the routine blood typing of about 4,000 persons. Detailed analysis showed that we were dealing with a characteristic, so-far unknown type of A blood corpuscle, /422 which was designated as A_3.

2) During an investigation of the families of these 6 persons from a group of 260 individuals, a total of 46 A_3 individuals and 3 A_3B individuals was found.

3) The most important serological peculiarities of these types are described. The main characteristic is the extremely low agglutinability. There is hardly a doubt that the case described by Fischer and Hahn, which dealt with a case of an extraordinarily weak A-receptor, was concerned with an individual of this type.

4) Investigation of the families show that the mode of inheritance of this characteristic agrees with the inheritance of a blood group characteristic which is due to an independent allelomorphic gene A_3, equal to the other blood group genes. It is, however, dominated by A_1 and A_2, just as A_1 is dominated by A_2. Therefore, the A_3 type forms a group or subgroup within the ABO blood group system.

5) The practical significance of the A_3-receptor lies in the fact that because of its weakness, it may be overlooked and may lead to erroneous blood typing. It could not be detected with 3 randomly selected commercial sera. As they relate to questions of parenthood, the practical consequences are

discussed. Assuming that the above-mentioned genetic theory is correct it is stressed that such an error could not lead to erroneous exclusion of fatherhood.

REFERENCES

1. Friedenreich, V.: Dtsch. Zeitschr. f. gerichtl. Med. 24: 167, 1935.
2. Friedenreich, V.: Klin. Wochenschr. 15: 310, 1936.
3. Friedenreich, V. and Zacho: Zeitschr. f. Rassenphysiologie 4: 164, 1931.
4. Fischer and Hahn: Zeitschr. f. Immunitätsforsch. 84: 177, 1935.
5. Hirszfeld: Weichhardts Ergebnisse d. Hyg. Vol. 15, 1934.
6. Landsteiner and Levine: Journ. of Immunol. 18: 87, 1930.